

MYOCARDIAL SCINTIGRAPHY WITH $^{99}\text{Tc}^{\text{m}}$ STANNOUS PYROPHOSPHATE

an experimental and clinical
study

Jan Lessem



Malmö 1982

Lund 1982



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by

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To Eva, Martin and Sarah

All the world is a stage and
all the men and women merely
players: they have their exits
and entrances: and one man
in his time play many parts,

Jacque's monologue As You
Like It, Act III
Shakespeare

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ABBREVIATIONS

AMI	— acute myocardial infarction
UAP	— unstable angina pectoris
AP	— anterior posterior position
LAO	— left anterior oblique
^{99}Tcm	— $^{99}\text{Technetium}^{\text{m}}$
PYP	— Pyrophosphate
^{201}Tl	— $^{201}\text{Thalliumchloride}$
CCU	— coronary care unit
ROC	— receiver operating characteristics
MUGA	— multiple gated acquisition study
mCi	— milli Curie
mBq	— milli Bequerel
IAS	— intensity added score
T	— transmural
SE	— subendocardial
Ca	— calcium

INTRODUCTION

During several decades one of the major death causes of the western world has been ischemic heart diseases (Moriyama et al. 1971, Stamler 1973). This is true even after the introduction of coronary care units (CCU), although death due to arrhythmias have decreased substantially (Hofvendahl 1971, Wilhelmson 1974), while only minor improvements have taken place in the treatment and outcome of cardiogenic shock. These mainly pharmacological achievements are also of rather recent date (Tuttle 1978, Waagstein et al. 1978). Of the patients admitted to the CCU due to persistent and severe retrosternal pain only about 50% develop an acute myocardial infarction (AMI), (Elmfeldt 1974) while 30% are discharged due to other manifestations of the ischemic heart syndrome, the most common being severe angina pectoris, while the remaining 20% have had other cardiac disorders such as pericarditis, myocarditis, valvular diseases, cardiomyopathy, arrhythmia of unknown etiology or in a non negligible number non cardiac diseases amongst which hiatus hernia plays an important role.

Of patients admitted with unstable angina pectoris (UAP), it has been shown that some later developed an AMI (Hultgren 1976) but difficulties have arisen when attempts to characterize these patients have been made.

New possibilities of salvaging reversibly damaged cells either by pharmacological (Marokko 1975, Nayler 1981) or surgical intervention (Hultgren 1976, Hurst 1979), have introduced the need for more precise, fast and reliable diagnostic tools. The time between the patient illtaken, final diagnoses and therapeutical intervention has become vitally important. At the same time the hemodynamic status of the heart and especially the left ventricle as an important parameter in the final prognoses has been more extensively recognized and focused on. For these reasons different non invasive methods for evaluating the status of the myocardium as to the degree of ischemia (Buja et al. 1975) and the hemodynamic changes during ischemic events have been developed. Among the most widely employed are the relatively new radionuclide methods (Ashburn et al. 1973, 1978, Strauss et al. 1977, Wagner et al. 1977). Attempts have also been made to use two or three dimensional echocardiography for these purposes (Nieminen et al. 1977,

Heikkilä et al. 1978, Horowitz et al. 1981), but so far no definite reliable results have emerged from these trials. Echocardiography proved itself a valuable tool when examining drug interference in these patients (Heikkilä et al. 1979). In spite of ECG-recordings the localization and size of the necrotic or ischemic area may be difficult to determine. Extensive ECG-mapping, as described by Marokko et al. will aid in these evaluations but may be influenced by several factors such as electrolyte imbalance, pericarditis, bundle branch block, obesitas and therefore its accuracy has been questioned. Coronary angiography as an investigation is not without risks. During the acute stages of the disease although it will supply information about the anatomical conditions of the coronary arteries, it may also fail in the presence of the coronary spasm syndrome (Maseri et al. 1977, 1981). Left ventricular angiography will yield information about the hemodynamic status of the left ventricle.

During the last decade new enzymes and enzyme determination techniques for excluding an AMI and for evaluation of the size of the necrotic area and the final prognoses of the patient have been developed. Creatine phosphokinase was described to be the safest and earliest responder (Shell et al. 1971, 1973, Nordlander 1976), and the iso-enzyme patterns with three different iso-enzymes of which one seem to be myocardial specific, have led to the evaluation of the method using myocardial bound CPK for quantitation of myocardial loss (Sobel et al. 1972, Klein et al. 1973, Sobel 1975, Ljungdahl et al. 1978). However not all data indicate that results from these methods are improving old diagnostic routines (Fex 1979, Bark et al. 1981). Furthermore not every laboratory has these techniques readily available and therefore uses the more traditional S-ASAT, S-ALAT, S-LD with or without iso-enzymes in the diagnostic procedure. Other methods being developed are techniques for measuring myoglobine in serum and urine (Willerson et al. 1976, Schersten et al. 1978) both probably early infarct responders.

Despite the afore mentioned improvements of the laboratory techniques, even the diagnoses of AMI let alone of UAP, may be difficult when ECG and enzyme responses are equivocal or the infarcts small in size. Therefore new diagnostic techniques have been developed, and among these are the previously mentioned radionuclide methods

(Parkey et al. 1974, 1975, Strauss et al. 1976, 1977, Hamilton et al. 1978, Botvinick et al. 1978). They are all non invasive and yield quantitative, qualitative hemodynamic and diagnostic information (Ritchie et al. 1978, Verba et al. 1979). Not until recently has it been possible to use these techniques in the acute stages of the disease as it seems to be necessary to examine patients with suspect ischemic heart disease either in the CCU or under continuous ECG monitoring or upon arrival in the emergency room, so that proper immediate intervention can be given if needed. With the development of the mobile gamma cameras, with or without an onboard computer, the bed-side diagnostics with radio-nuclides have become more readily available to the physician. Difficulties and discrepancies in the interpretation of myocardial scintigrams especially with $^{99}\text{Tc}^{\text{m}}$ -pyrophosphate has lately led to a debate, summarized by Bianco in 1981, of the value of these diagnostic procedures. Only a few investigations where the observers were blinded when evaluating the scan (Cuaron et al. 1979, 1980, Atwood et al. 1981) have been published trying to clarify these questions.

OBJECTIVES OF THE PRESENT INVESTIGATION

The purpose of the present investigation is to try to answer the following questions in an experimental animal study and in a clinical study of 635 patients.

- 1) Does in animals of different species with induced myocardial infarction, a radionuclide accumulation occur in the necrotic area?
- 2) What is the specificity, sensitivity and efficiency of the radionuclide methods in the diagnoses of AMI?
- 3) Do different degrees of ischemic heart disease exhibit different patterns of radionuclide accumulations?
- 4) Do different patterns of radionuclide accumulation indicate different prognoses?
- 5) Is it possible to diagnose AMI earlier after onset with radionuclide methods than with ECG and serum enzymes?
- 6) How reliable are scintigraphic results in the diagnosis of AMI when judged blindly by several interpreters?

HISTORICAL REVIEW

The history of cardiovascular nuclear medicine is intimately connected with the development of nuclear medicine in general, and the desire in finding less traumatic diagnostic procedures than the invasive method. Among the pioneers to describe the usefulness of radionuclides in determining hemodynamic cardiac events was Nylin, when he showed the feasibility of cardiac measurements and cerebral blood flow studies using radionuclides. The earliest methods for a more clinical use was radiocardiography, a method by which Prinzmetal in 1949 could monitor the time course of a radioactive tracer through the heart, using a single unshielded Geiger-Müller tube measuring the radioactivity over the point of maximum cardiac impulses, following an intravenous injection of ^{24}Na . These studies were performed mainly in children with congenital heart diseases. These hemodynamic studies were initially an application of the indicator-dilution techniques, and observations obtained, yielded time activity curves. Later with the development of a gamma camera by Anger, a procedure similar to contrast angiography was adhered to. Fast hemodynamic events became recordable. Qualitative assessments of shunts and of the function of the left ventricle were performed. These techniques are now a routine in many areas of investigation and used in both large and small hospitals. Gated blood-pool studies, where recordings were made during one whole or proportions of many cardiac cycles and where recording principally is triggered by the R-wave, with determination of ventricular wall motion and both global and regional left and right ventricular ejection fractions are part of the routine control of patients with severe heart diseases and these methods have been described in detail (Strauss et al., Shelbert et al., Ritchie et al., Hamilton et al., Verba et al., Rigo et al., Planiol et al., Adams et al.). More sophisticated computer programs have made these hemodynamic investigations safer than they previously were (Verba et al. 1979, Adams et al. 1980).

When evaluating ischemic heart diseases other techniques have been employed and two different directions have been followed. One direction using substances mainly accumulating in normal myocardium, in scintigrams reflected as a defect at the site of the necrotic or ischemic area — often referred to as cold spot scanning. The other way has been to utilize substances mainly accumulating in necrotic or ischemic

myocardium — so called hot spot scanning — TABLE I —. Finally metabolic scanning agents have been investigated.

TABLE I

Tracer	Half-life	Energy level-keV	Labelled to	Special features
Potassium-38	7,7 min	511	—	positron emitter
Potassium-43	22,2 hours	373, 618	—	perfusion studies
Rubidium-81	4,7 hours	190, 511	—	perfusion studies
Rubidium-82	1,25 min	511, 776	—	positron emitter
Cesium-129	32,0 hours	375, 415	—	perfusion studies
Cesium-131	9,7 days	31 — 36	—	perfusion studies
H ₃ N-13	10,0 min	511	—	positron emitter
Thallium-201	74,0 hours	80, 135, 167	—	perfusion studies
Carbon-11	20,5 min	511	—	positron emitter
Oxygen-15	2,0 min	511	—	positron emitter
Technetium-99m	6,0 hours	140	tetracycline pyrophosphate glucoheptonate	infarct avid
Gallium-68	68 min	511	— EDTMP	infarct avid

The table summarizes some of the most important and used isotopes in nuclear cardiology.

COLD SPOT SCANNING

It was first shown by Love et al. in 1954 that substances accumulating in normal myocardial tissue were potassium and potassium analogues. These substances are believed to reflect the regional myocardial perfusion pattern. Sapirstein et al. in 1965 showed that potassium is largely extracted by the myocardium during the first circulation, and the fraction of the indicator extracted, when evenly mixed with the blood-pool at the ventricular outlet, is proportionally distributed to the different organs reflecting the cardiac output, a finding, confirmed by Maseri et al. in 1976. Potassium or any of its analogues will therefore after equilibrium reflect the potassium pool and also constitute a measurement of the myocardial mass. Potassium and its analogues are furthermore taken up by myocardial cells but the function of myocardial capillary blood flow as well as by active transport across the cardiac muscle, cellular membrane. These described mechanisms are the basis for the use of potassium isotopes, and if coronary occlusion is present the distribution of potassium will be affected both proportionally with the cardiac output and over the cell membranes leading to diminished potassium extraction in ischemic areas secondarily giving rise to cold spots or more accurately perfusion defects. Even if radioactive potassium uptake is thought to be mainly flow dependent, and regional myocardial uptake is a reflection of regional flow, several factors may alter the extraction or uptake pattern at the membrane level, at a rather unchanged and constant flow. Changes in energy utilization in membrane ATP-ase may lead to significant changes in cation extraction and uptake patterns. Based on these principles Carr and coworkers in 1962 performed several animal experimental studies where myocardial infarction was induced surgically. In 1964 these experiments led to the suggestion that two potassium analogues ^{86}Rb and ^{131}Cs might be clinically applicable. Following these initial experiments further studies were done with ^{42}K , ^{43}K , ^{129}Cs and ^{81}Rb . TABLE II summarizes the physical properties for potassium-43 and some of its analogues.

The disadvantages of prolonged examination time when using a rectilinear scanner with these radionuclides was eliminated when Leibowitz et al. in 1975 introduced ^{201}Tl for myocardial perfusion

TABLE II

Radioisotope	Half-life, hours	Energy levels, keV	Whole body radiation dose (mrads/mCi)
Potassium-43	22,2	373, 619	622
Cesium-129	32,1	375, 415	240
Rubidium-81	4,7	190, 511	222
Thallium-201	74,0	80, 135, 167	70
Cesium-134m	2,9	128	48

Some of the available and used potassium analogues. ^{201}Tl is the isotope with the least whole body radiation dose.

imaging studies, as ^{201}Tl has a major low energy peak which was suitable for the gamma cameras. Simultaneously Bradley-Moore in 1975 could show following an intravenous administration of ^{201}Tl , that it was primarily distributed intracellularly and was similar to potassium in its myocardial distribution. A year later, in 1976, Gehring and Hammond proved that Tl behaved biologically similar to potassium, because it has an ionic radius in between Ca^{2+} and Rb^{+} . The Tl is a cyclotron produced radioisotope and produced by irradiation of a natural Tl target with an external proton beam. The nuclear reaction is $^{203}\text{Tl} (\text{T}, 3\text{n}) ^{201}\text{Pb}$ and thus ^{201}Pb with a half-life of 9,4 hours is the parent of ^{201}Tl . After letting ^{201}Pb decay, ^{201}Tl is separated from the remaining ^{201}Pb by column chromatography. However this way 1% of ^{200}Tl with a half-life of 20 hours and emitting gamma rays of 368keV and 0,5% of ^{202}Tl with a half-life of 12 days emitting gamma rays of 440keV are present. These amounts and physical characteristics are however too small to influence clinical studies as the remaining 98,5% of ^{201}Tl dominates. It decays by electron capture and emits mercury X-rays of 69 to 82keV and photons of 135 and 167keV and has a half-life of 73 hours. It was shown by Wackers et al. in 1976 to be the best suited potassium analogue to use in combination with a gamma camera. During the last years several reports have appeared describing the usefulness of ^{201}Tl myocardial scintigraphy in both the diagnoses of AMI and in detecting coronary ischemia at exercise (Wackers et al. 1976, 1977, Hamilton et al. 1977, Staniloff et al. 1980). Thus it was justified to use ^{201}Tl as a potassium analogue in this investigation and utilize it for non invasive determinations of regional myocardial perfusion. When used in the clinical routine it has to be borne in mind that infusion of glucose and insuline may lead to an increased uptake of radioactive

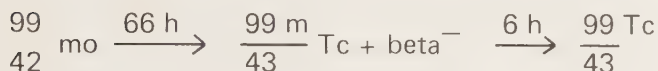
potassium or its analogues by the myocardium. Digitalis as well as commonly employed diuretics may change the myocardial potassium balance and thus the uptake pattern. Cardiovascular drugs that have been shown to decrease the radioactive potassium uptake are beta adrenergic blockers e. g. propranolol (Kortin et al. 1976). Electrolyte changes in Mg^{2+} , as an interaction between potassium, Mg^{2+} and even Ca have been described (Dyckner in 1979, Dyckner et al. 1981), besides potassium changes may effect the uptake pattern. Cautiousness is therefore well advised when evaluating the obtained results, independently of which potassium analogue is used.

HOT SPOT SCANNING

Another approach to myocardial infarction imaging has received growing interest since the early 1960:s and this is the so called hot spot imaging which refers to substances accumulating in necrotic myocardium thus visualizing regions of the infarct or ischemic myocardium as positive zones of increased concentration of a radioactive tracer. The earliest reports on such substances were concerned with iodine where Dreyfuss and coworkers found increased activity in areas of infarcted myocardium in patients several days and weeks after infarction. Later studies casted a shadow of doubt as to whether the concentration of radio iodide in infarcted myocardium was sufficient to allow external good imaging and Louvranij provided a dissatisfactory ratio between infarcted and non infarcted myocardium and therefore radio iodide was written off as a suitable agent for infarction imaging. In further experimental studies mainly in dogs, it was shown that mercury isotopes such as ^{203}Hg in chlormerodrin or hydroxymercury fluorescein accumulated to some degree, depending on the age of the infarction and the necrotic myocardium, and gave satisfactory scintigrams (Carr et al.1962, Maleck et al.1967, 1971, Hubner 1970, Ramanathan et al.1971). However no success was achieved with these substances when they were used in the clinical studies. Trials with ^{203}Hg labelled substance turned out negative results (Carr et al. 1963). ^{203}Hg has a long half-life and non-penetrating radiation and accumulation in the kidneys which only allows smaller doses than in animal studies to be administered to humans and thus these substances were all abandoned for routine clinical use.

While working with mercury type substances Malek and coworkers found that if using tetracycline compounds they could detect infarcts due to the property of fluorescence, which the tetracyclines have. It also enabled them to follow stages in the development of an infarct. The problem was to find a suitable labelling radioisotope which could be used for external detection. It was clear at that time that rapid clearance of the radionuclide from the blood and other tissues around the heart was essential so that the background activity could be minimized. High retention of the pharmaceutical in the infarcted areas and low retention in normal myocardium were two necessary properties for the suitable agent to possess. It was not until a stable complex with

$^{99}\text{Tc}^{\text{m}}$ and tetracycline was synthesized using a stannous chloride reduction method that such a compound was found (Dewanjee et al. 1976). With $^{99}\text{Tc}^{\text{m}}$ an ideal radioisotope with a half-life of 6 hours gamma ray energy of 140 keV was introduced. Compounds of Tc are known to exist in valence stages from +7 to -1. The most stable is the pertechnetate ion TcO_4^- . The interesting medical radionuclide is formed by beta decay of molybden-99.



The molybden isotope is obtained either as a fission product, or produced when neutrons are allowed to bombard molybden in a nuclear reactor. $^{99\text{m}}\text{Tc}$ is obtained as an eluate from the parent isotope. Holman and coworkers in 1973 showed that $^{99}\text{Tc}^{\text{m}}$ tetracycline was a useful radiopharmaceutical for infarct imaging in animals, but later pointed out in 1974, 1975 that infarct imaging in humans was only possible 24 hours after injection of the substance. It was hypothesized that tetracycline binds reversibly to denaturated macromolecules such as RNA and DNA in necrotic cells. At the same time it was suggested that tetracycline accumulation mirrors Ca-concentration, indicating a lack of specificity, and tetracycline was also shown to accumulate in tumors and focal infections.

In search for the ideal pharmaceutical for infarct avid imaging other $^{99}\text{Tc}^{\text{m}}$ complexes have been utilized. One such substance where initial studies were encouraging was $^{99}\text{Tc}^{\text{m}}$ glucoheptonate (Fink et al. 1974). Scintigrams in dogs became positive as early as 6 hours post infarct, but best results were obtained 24 hours after the infarction had occurred (Rossman et al. 1974). When a clinical study was conducted by Rossman and coworkers in 1975, $^{99}\text{Tc}^{\text{m}}$ glucoheptonate seemed to be relatively specific for identification of acute infarction but was somewhat insensitive with a high rate of false negatives. Recently Jacobstein and coworkers showed renewed interest in $^{99}\text{Tc}^{\text{m}}$ glucoheptonate as a potential infarct imaging agent. Later studies with $^{99}\text{Tc}^{\text{m}}$ gluconate have also showed some promising results.

Parallel with the development of the glucoheptonate compounds $^{99}\text{Tc}^{\text{m}}$ labelled phosphates were experimented with for infarct avid scanning. Bonte et al. in 1973 described that pyrophosphate labelled with $^{99}\text{Tc}^{\text{m}}$ yielded positive scintigrams in dogs with experimentally induced infarctions. In 1974 and 1975 Parkey and coworkers showed that this was also the case in patients with an AMI. These compounds were all bone imaging agents (Subramanian 1972, 1975) where they had been universally tried both in experimental and clinical settings, and well studied before used for cardiac purposes. The phosphates were found to accumulate in necrotic skeletal areas. When first utilized in cardiological diagnostics it was believed to be a myocardial infarction specific agent, but the specificity has recently been challenged and several other entities have been reported to accumulate the pyrophosphate — TABLE III —. The mechanism behind the pyrophosphate loca-

TABLE III
Disorders where $^{99}\text{Tc}^{\text{m}}$ -PYP has been found positive, except AMI

- Unstable angina pectoris
- Cardiomyopathy
- Left ventricular aneurysms
- Rib fractures
- Cardioversion
- Myocardial contusion
- Chagas disease

lization in irreversibly damaged myocardial cells remain obscure, but it has been speculated that behind the accumulation an affinity to hydroxy-apatite crystals plays an important role. D’Augustino and Shiga in 1970 showed the localization of Ca in a mitochondria in the necrotic myocardium and also suggested that this Ca was incorporated into a crystalline structure, which they believed to be hydroxy-apatite. Shen and Jennings in 1972 also supported this theory by showing that within the intramitochondrial dense bodies, Ca uptake was an active process into these bodies consisting of Ca-phosphate. Bloom and Davies later also proved the presence of vast amount of Ca intramitochondrial in isoproterenol induced myocardial necrosis. However it was Jung and his collaborators, in 1973, who managed to show that hydroxy-apatite crystals had a great affinity for pyrophosphate, in fact greater than for

disodium ethan-1-hydroxy-1-1-disphosphonate (EHDT) and disodium chlormtehylenedisphosphonate (Cl₂MDP). During the process of binding pyrophosphate, ortophosphate was released and it was shown that pyrophosphate had no appreciable effect on the distribution and the fluxes of Ca. The reason for the differences between the compounds may be that EHDT and Cl₂MDP occupied more sites than did pyrophosphate. These background studies justified Bonte and coworkers to use pyrophosphate as an apatite labelling tracer, postulating the existence of hydroxy-apatite crystals intramitochondrial in the necrotic myocardium. Buja and coworkers have later shown this to be one of several uptake mechanisms.

In search for explanation of the pyrophosphate uptake electron-microscopic studies have been performed. These have demonstrated constant alterations of the mitochondria in the myocardial cells from the same areas, that demonstrate abnormal Ca uptake. Electrone dense spicules were seen, that were believed to represent apatite crystals. The electrone dense granular deposits were representing a Ca-phosphate precipitation of a noncrystalline form, as well as an amorphous deposit, or they were aggregates of denaturated mitochondrial protein. Interestingly enough the time course of the electrone dense deposits parallel the intensity of the scintigrams. Maximal deposits, were found in infarcts 1-2 days old when also scanning yielded the best results. Smaller quantities of deposits were seen on the 7th day, almost disappearing 2 weeks after the infarct had occurred and rarely yielding positive scintigrams. The deposits were almost exclusively found in the periphery of the infarcted area and the absence of these Ca-deposits in the central infarct region may explain the dough-nut pattern with a central spare zone.

However Dewanjee and Khan in 1976 suggested a complementary localization path-way after having shown that pyrophosphate accumulated not only in mitochondria but also to some extent in other subcellular organisms. They were able to show that ⁹⁹Tc^m- pyrophosphate was concentrated in the same infarct areas as the ⁴⁵Ca ion, but to a much greater degree and also that ⁹⁹Tc^m chelates such as pyrophosphate were mainly protein bound, and thereby confirmed data by Krishnamurthy et al. in 1974, showing that pyrophosphate chelated with reduced Tc-ion mainly was bound non specifically to serum albumine or

fibrinogene and gammaglobulines. They also observed that in comparison to ^{45}Ca , $^{99}\text{Tc}^{\text{m}}$ pyrophosphate was removed earlier from the blood-pool due to skeletal uptake thus concluding that it was the better infarct imaging agent of the Tc-chelates. From their observation that there existed a correlation between protein binding capacity and localization of $^{99}\text{Tc}^{\text{m}}$ -pyrophosphate in infarcted myocardium, was suggested that denaturated macromolecules hosted Tc-chelates to form polynuclear complexes. From these observations it can be seen that the sub-cellular mechanism for pyrophosphate binding to irreversibly damaged myocardial cells still is not very accurately understood. At the same time Buja et al. in 1976 studied the correlation of the pyrophosphate uptake and the necrotic areas on a morphological histological basis and found that most of the pyrophosphate accumulated in the outskirts of the necrotic areas, but could not find any correlation of the uptake to enzyme contents of various parts of the myocardium. It has been speculated that the Tc-ion alone may play a role in the uptake mechanism, and form new chelates with polyanionic substances that ought to be present in the infarcted myocardium, Poe (1977) drew such a conclusion. It is unknown with what other ions such an interacting could take place.

METABOLIC AND POSITRON SCANNING

TABLE IV

Agent	Half-life	Use
H ₂ 150	2,03 min	Diffusable use
⁷⁷ Kr	71,4 min	Diffusable use
¹¹ CO	20,4 min	Blood pool agent
⁶⁸ Ga-RBC:s	68 min	Blood pool agent
²⁸ K	7,71 min	Perfusion studies
⁸² Rb	1,25 min	Perfusion studies
¹¹ C-palmitate	20,4 min	Perfusion and metabolic studies
¹³ NH ₃	10,0 min	Perfusion and metabolic studies
⁶⁸ Ga-EDTMP	68,0 min	Infarct avid studies

The most used positron emitters.

Other substances of interest have been positron emitters –TABLE IV– where a nuclear proton changes into a positron and a neutron. The ejected positron is combined immediately with an electrone and these two particles undergo annihilation where the energy associated with the masses of the positron and the electrone is 1,022 MeV. This energy is divided equally between two photones separating from each other with an angle of 180 degrees, leaving each photone with an energy of 511 keV. One of these positron emitters that has been used is ¹³N in the form of ammonia, which physiologically behaves much like potassium. The high energy level requires specific instrumentation, a so called positron camera system described by Harper et al. in 1973, Budinger et al. in 1977, Hoop et al. in 1973. In combination with a positron camera system where the annihilation photones are detected by two opposite radiation detectors, tomographic pictures of e. g. the heart can be obtained (Walsh et al. 1976, 1977) and with a computer program by Atkins and coworkers in 1977, the out of focus data can be corrected for, resulting in reconstruction pictures of high quality. Positrons such as ¹¹C have been attached to different fatty acids (Poe et al. 1975, 1976), giving the opportunity to follow the metabolic event in a normal and diseased myocardium. Burton Sobel's group in St Louis has begun to utilize positron imaging in evaluating the possibility of determining metabolic ischemic events in the myocardium. The utilization of glucose by the myocardium can be accurately studied by using both positron and non-positron labelled fatty acids and these techniques may eventually become clinically more useful for mainly intervention studies.

EXPERIMENTAL STUDY

AIM

In order to investigate the clinical utility of ^{99}Tcm pyrophosphate several animal experimental studies were performed. The major objectives of these studies were to try to answer how pyrophosphate accumulated in necrotic myocardium, after experimentally induced infarction. Before clinical trials could commence or data found at clinical trials could be properly interpreted it was important to know in which type of myocardium the major uptake was to be found, especially whether it was in the infarcted areas or in the border zone.

MATERIAL

Seventy-two female albino rabbits weighing 2.0-4.5 kilograms, 42 female Sprague-Dawley rats weighing 200-250 grams and 5 mongrel dogs weighing 17-37 kilograms were included in the study. The animals were divided into three groups according to the method of infarct induction and imaging —Table V —.

TABLE V

Group	Number species	Method of infarction	Method of examination
A	39 rats	LAD ligation	Gamma camera
	2 rats	No infarction	Gamma camera
	1 rat	No surgery	Gamma camera
B	5 dogs	LAD ligation	Video scanner
	6 rabbits	LAD ligation	Video scanner
C	66 rabbits	Isoproterenol injections	Video scanner

The animal material, divided into groups after the way of producing the infarction.

METHODS

Surgical procedures

Group A consisted of 42 rats and in 39 of these myocardial infarction was induced by a permanent ligation of the left descending artery according to the technique described by Bajusz in 1963 and modi-

fied by Pohlmeni and coworkers in 1975. These rats were anaesthetized with ether, the heart was exposed and exteriorized through an incision between the 4th and 6th ribs on the left thoracic side. The artery was ligated approximately mid-way between the base and the ventricular apex. The thoracic wound was then rapidly closed using metal clips instead of sutures. The procedure described lasted 3-5 minutes and no artificial respiration was required, as surviving rats began to breath almost immediately after surgery. Of the 42 rats, 10 died during or immediately after the surgical procedure. The small diameter of the rat coronary artery and its intramyocardial localization makes it almost impossible to visualize the vessel during the surgical procedure and thus the created infarcts, although definitely transmural, varied in size. This technique due to the approximation when defining the length of the coronary artery exhibits difficulties in reproducibility. Three rats were used as controls, and of these 1 served as a normal control and 2 were sham operated on with skin incisions and rib cracking but no myocardial surgery performed. The pericardium was in all rats incised at the site of the ligature, but at no other place. No sutures were placed in the pericardium.

Group B included 6 rabbits and 5 dogs where the technique for coronary ligation described by Myasnikow in 1961 was used. The animals were anaesthetized during the whole surgical procedure with mebumal sodiumchloride (Abbot Laboratories, USA). An incision was made on the left thoracic cavital side and the position of the heart recognized. An incision between the 5th and 7th ribs was followed by a small incision of the pericardial sac and then the left descending coronary artery was visualized at its root, and a ligature was placed here, after which the wound was closed. The animals required sufficient artificial respiration to survive, and were monitored with continuous ECG recordings during the surgical procedure. Two hours post surgery all dogs and rabbits were injected with 10 mCi (350 mBq) $^{99}\text{Tc}^{\text{m}}$ pyrophosphate (Diagnostic Isotope Inc., USA), equally to 8–10 mg pyrophosphate. No imaging procedure was carried out in the dogs, while the 6 rabbits were scanned 48-72 hours post ligation. A rectilinear scanner was used and AP and LAO 30° positions used. One hour after the radionuclide injection open heart biopsies were taken from the dogs without introducing a catheter. Pieces were extracted with specially constructed biopsy forceps. The biopsies were taken close to

the ligature, and in the border zone, and finally far from the areas supplied by the artery ligated —Fig. 1—. This procedure was then repeated

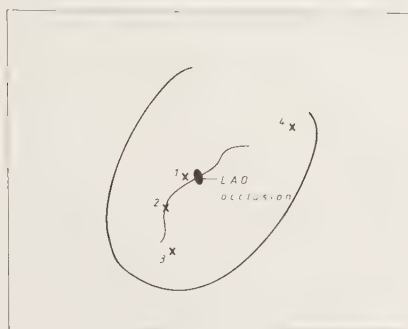


Fig. 1

A schematic picture of the ligated dog artery, demonstrating the areas where biopsy were taken.

ted with an interval of 1 hour for another 5 hours. Between every biopsy occasion the chest wound was temporarily closed with metal clips which did not reach the myocardium. They were easily removed and reused. In one of the dogs 1,5 mCi ^{201}Tl (Philips Duphar, the Netherlands) was administered simultaneously with ^{99}Tcm pyrophosphate. Otherwise no changes were made. After the final biopsies the animals were sacrificed and pieces from the infarcted areas, border zones and distal areas were taken, after visual judgement, and a post mortem examination performed.

Group C was comprised of 66 rabbits where myocardial infarction was induced by subcutaneous or intravenous administration of isoproterenol in 46 of the animals according to the technique described by Rona et al. in 1959. The catecholamine was given as a repeated injection over a period of time, varying from 12 to 48 hours. Each time 0,2 mg was given until the rabbit had received 3,0 mg isoproterenol per kilo body weight. When administered intravenously isoproterenol was given through a butterfly cannula in the vein of the ears. All rabbits were anaesthetized for every intervention with mebumalsodiumchloride. The remaining 20 rabbits served as normal controls and therefore in none of them isoproterenol was given or any surgery performed. Before scanning all rabbits were again anaesthetized.

All animals in group B and C were given 4,0 mg pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7 \times 10\text{H}_2\text{O}$) $\times$ C3, labelled with 2–3 mCi $^{99}\text{Tc}^{\text{m}}$ (Techne-Scan, Byk-Mallinckrodt, USA) and examined during different time intervals post injection and post surgery. After conclusion of an experiment a post mortem examination was performed.

Investigational techniques

The rats allocated to group A were imaged in the AP and left lateral position using a 2,3 mm Tungsten pin-hole collimator mounted 18 cm from the crystal face of a gamma camera -Pho Gamma IV, (Searle Radiographic, USA). The animals were positioned close to the pin-hole so that a magnification factor of 6.10 at the crystal face was obtained. Imaging procedures were carried out under ether anaesthesia. Data was collected in a digital form in a 128 x 128 matrix and processed with an Ohio nuclear 160 Data System (Ohio Nuclear, USA). Each scintigram was judged as to the presence and intensity of an uptake. The intensity was arbitrarily graded 0-4, where 0 represents no uptake, 1 faint uptake as compared to the sternal and rib uptake, 2 an uptake less intense than the bone uptake, 3 an uptake equalling the intensity of the skeletal uptake and 4 represents an uptake stronger than the bone uptake. Each rat was injected 60 minutes after coronary artery ligation with 3 mCi $^{99}\text{Tc}^{\text{m}}$ pyrophosphate (Squibb Laboratories, USA) equally to 0,168 mg pyrophosphate. The injection was done in the tail vein.

In vivo studies were carried out in 9 animals of whom 6 had an infarct, 1 was sham operated on with only a skin incision, 1 sham operated by breaking a rib and finally 1 served as a normal control –TABLE VI—.

TABLE VI

Number	Operation	Study
6	LAD ligation	In vivo
12	LAD ligation	In vitro
1	skin incision	In vivo
1	rib cracked	In vivo
21	LAD ligation	No scanning
1	No operation	Normal control

Table VI illustrates the procedures in the rat population.

Imaging procedures were started 60-90 minutes after injection, collecting 100.000 counts in each image. The imaging procedure was repeated 2, 3, 4, 24 and 72 hours after surgery. In vitro studies were performed in 12 animals with infarcts. The heart was excised while beating under ether anaesthesia 3 hours after injection of the radioactive agent. The heart was placed in saline and imaged at the AP projection collecting 7.000-10.000 counts. The area of interest was moved over the necrotic myocardium towards the border and finally to normal myocardial areas and the counts accumulated in the computer. Under these conditions counts densities (counts per pixel — that is to say counts per square of the oscilloscope matrix, a pixel being the smallest square—) represents approximately the average activity concentration in the tissue assuming equal thickness. Weighed tissue samples from the infarcted and normal regions were assayed for their radioactivity content (after perfusion with physiologic saline solution and dried on a paper sucking up the fluid) in a well counter (Hewlett-Packard, USA).

Appropriate correction for physical decay was made as well as correction for the background activity and normalization to blood-pool levels obtained at the time of sacrifice. Two measurements were obtained for each sample and the mean value was used for calculation. Each piece was counted for 1 minute.

Twenty-one rats were subjected to coronary artery ligation but no radionuclide studies. The hearts were excised at the post ligation interval comparable with the scanning time, but also studied one week after ligation. Sections were stained with hematoxylin and eosine and with a fuchsin stain described by Poley et al. in 1964. All hearts were sectioned in a plane passing through both ventricular free walls and apex including normal border zone and infarcted regions of the myocardium.

In group B and C scintigraphy was performed with a video-scanner (El-scint, Israel) 45–60 minutes after radionuclide injection. The video-scanner is a rectilinear scanner consisting of a moving detector imaging system. The detector is a NaI (TI) crystal with photomultiplier tubes and a focusing collimator constructed of lead in order to limit the field of view.

In the rabbits in group B and C, scintigrams were recorded in AP, LAO 30° and left lateral position and were evaluated as to the presence or absence of hot spots. No intensity grading of an uptake was performed. In the rabbits whole-body scintigraphy was also performed — Fig. 2 —.



Fig. 2
A whole body scintigram in a rabbit with a myocardial infarction.

One hour after scintigraphy the rabbits were sacrificed, the heart perfused with saline and divided visually into areas of normal myocardium and infarcted areas. Biopsies from rib, liver, spleen and lung were also taken. Together with the myocardial pieces these biopsies were counted for the content of radioactivity in a well counter (General Electric, USA) and histological examination was performed by an experienced pathologist (Ass. Prof. Alf Rausing, Malmö).

RESULTS

Group A rats were divided into different subgroups. In vivo imaging studies showed infarct localization, but interference from the traumatized chest wall made the estimations of localizations somewhat difficult on these rats, but the intensity of an uptake increased over the time period reaching maximum at 24–48 hours post surgery — Fig. 3 —. However when examined again at the 72 hour interval, the uptake had totally disappeared in the in vivo examined hearts — Fig. 4 —. In the 2 sham operated rats no pyrophosphate accumulation was registered. The rat with the broken rib showed a distinct pyrophosphate accumulation, as expected in the area of the broken rib — Fig. 5 —.

Count density

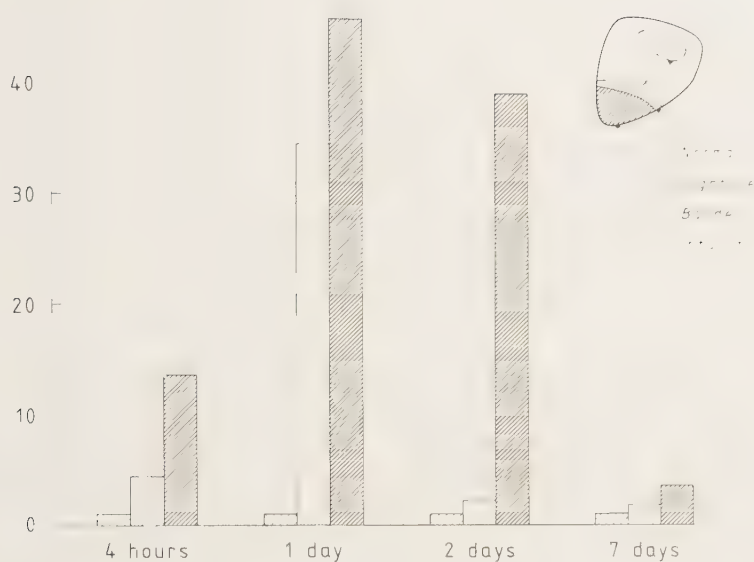


Fig. 3

The rat model, demonstrating the highest radioactivity in the infarcted areas 24 hours post surgery.

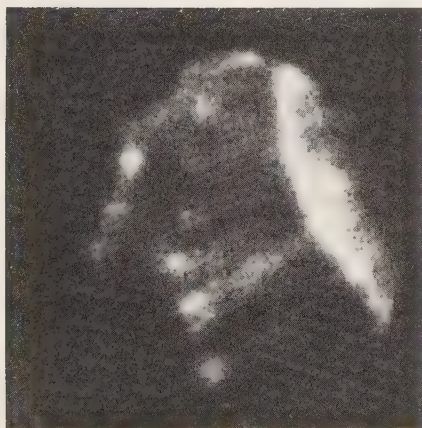


Fig. 4

A rat ligated 72 hours before scanning. No uptake in the myocardium was present at this time.



Fig. 5

The rat with broken rib. Note the high density uptake over the rib area.

When the intensity of the scintigrams were compared between excised hearts and those examined in vivo the strongest intensity was found in the excised hearts. We found a substantial uptake as early as 1–2 hours post ligation with a low grade intensity slowly increasing to a maximum intensity at 24 hours and then decreasing over a period of another 48 hours being almost absent at 72 hours post surgery. The increase of activity in the infarcted areas compared to normal was apparent and similar with both types of measurements previously described –TABLE VII–.

TABLE VII Tissue activity ratios: infarct/normal

Time after infarctions	Excised heart Activity/g	Counts/pixel	Ratio Tissue data image data
1 hour	27.8	15.1	1.84
	5.9	13.6	0.43
	7.3	6.6	1.10
Mean	13.7–7.1 *	12.7–2.5 *	
24 hours	74.6	38.6	1.93
	46.4	46.5	1.00
	16.7	10.3	1.62
Mean	45.9–16.7*	32.7–13.8 *	
48 hours	18.0	8.1	2.22
	47.0	20.3	2.32
	21.9	9.6	2.28
Mean	29.3–9.3 *	16.6–9.7 *	
72 hours	2.1	3.3	0.63
	2.3	2.6	0.88
	6.4	3.7	1.73
Mean	3.6–1.1 *	4.1–0.4 *	
			1.50–0.66 *

* Standard error of mean.

The highest ratio between normal and infarcted areas were found 24–48 hours post surgery. At 72 hours however the smallest ratios were found between these areas. Interanimal comparison yielded smaller variations than did intraanimal comparison. The data in the table shows that the ratios from tissue counting data average about 50 % higher. This is consistent with the fact that the apex of the heart where the infarcts often were present probably has a thinner muscular wall than the rest of the heart as seen on the AP projection, which means that the count per unit area will be smaller than the count per unit weight. The profiles across infarcts do not indicate any increased activity in a border zone —Fig. 6 —. When histologically examined already after 1 hour, the injured tissue was oxinophilic and sharply delineated whereas at 24 hours necroses of myocardial cells were evident and became more pronounced at 48 hours. Both at 24 and 48 hours an inflammatory infiltrate consisting of polymorph nuclear leukocytes was present. Interestingly enough it was found that 72 hours after ligation the cells in the infiltrates were mainly mononuclear cells, and there was evidence of partial replacement of necrotic cells by connecting tissue cells which were even more pronounced 24 hours later. One week after ligation all the necrotic cells had been replaced by connecting tissue scar. No rats were scanned at one week after ligation.

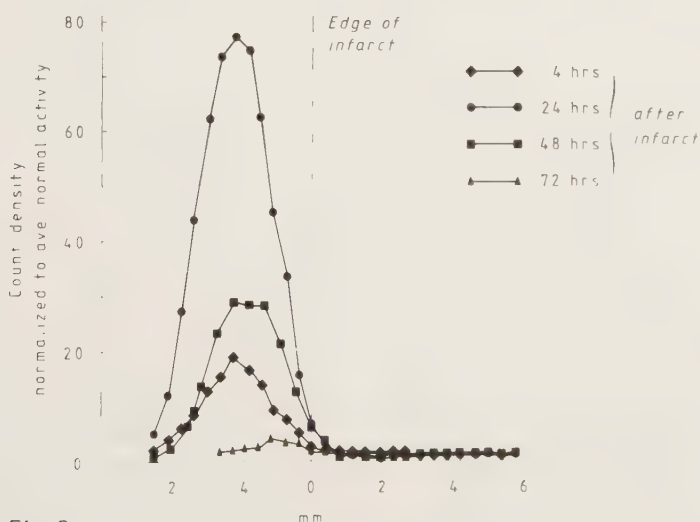


Fig. 6
Profiles across the infarcts in four animals at the time intervals studied. The low activity at 72 hours post ligation is apparent.

Group B comprised 6 rabbits had 5 dogs in whom the left descending coronary artery had been permanently ligated at the root of its exit from the aorta. Only the 6 rabbits were scanned, while biopsies from the heart only were taken from the dogs. The 6 rabbits were scanned 48–72 hours post ligation. They all showed a pyrophosphate accumulation in the areas where the infarct was created. Three had apical uptake while the other 3 showed an antero-lateral accumulation. At autopsy all 6 rabbits showed transmural myocardial infarction, covering both the anterior and apical part of the left ventricle. Histology showed heart muscle necrosis and polymorph nuclear leukocytes cell infiltration. No connective scar tissue was detected. It was found that the radio activity was high in the necrotic areas but relatively close to the activity of the border zone, while normal myocardium showed substantially less activity. In the dogs the radionuclide was injected 2 hours post surgery and 1 hour later the first biopsies were taken. Myocardial biopsies were then continuously taken with an interval of 1 hour for another 5 hours. The biopsies were taken from the area of the ligature, distal to the ligature, from the tissue judged as developing infarction and from the border of this tissue as well as from normal myocardium. In all 5 dogs the procedure was monitored by ECG and when the infarction was created the ECG changed, and ST-segment elevation of depression and T-wave inversion was developed, compatible with a pattern of AMI. Ventricular extrasystolies were also encountered. The tissue radioactivity was measured and corrected for background activity and tissue weight. In all animals $^{99}\text{Tc}^{\text{m}}$ activity turned out to be greater in the infarcted areas than in the border zone or in the normal myocardium. At 1 hour post injection (3 hours post surgery) normal myocardium had highest content of radioactivity but 2–4 hours later infarcted areas had much higher radioactive values.

One dog was also injected with 1,5 mCi ^{201}Tl before any biopsies were taken. Besides this the same procedure was followed. The Tl content was there also calculated parallel with $^{99}\text{Tc}^{\text{m}}$ pyrophosphate content and it was found that in this dog the normal myocardium exhibited the highest Tl content while the infarcted area contained a very minimal amount of Tl. The border zone content was almost identical to the normal myocardium (554 808 c/gm/m normal area as against a border zone value of 557 787 c/gm/m). Evaluating biopsies from the same areas in the same animal, the border zone contained more radioactive

pyrophosphate than did normal myocardium but less than infarcted areas. The two findings with different isotopes were thus superimposed and supported each other.

In the 46 rabbits in group C the trial to induce myocardial infarction by isoproterenol injection was successful in 35 (76 %) while 6 developed a toxic myocarditis and 5 showed normal histological pattern. When scanned, the 5 without infarct showed no pyrophosphate accumulation while the other 41 exhibited scattered pyrophosphate uptake. The infarct after isoproterenol injection were in all 35 rabbits wide spread. They were often very small in size and characterized by a round cell infiltration and a large number of fibroblasts, a finding also observed by Leszkowsky et al. in 1967, and Ostadal et al. in 1968. There was no possibility to separate between rabbits with infarction and rabbits with myocarditis when examined in vivo with pyrophosphate. In none of the 20 normal control rabbits did pyrophosphate accumulate abnormally.

DISCUSSION

Myocardial infarction in animals can e. g. be induced by either ligation of the coronary artery as described for rats by Selye in 1959, Bajusz in 1963 or in dogs as proposed by Myasnikow et al. in 1967, or by administering isoproterenol subcutaneously or intravenously as shown by Rona et al. in 1963, Kobayakawa in 1972 and Milei et al. in 1976, 1978. The finding in this series of experiments well coincide with and confirm all these previous reports. However when the infarctions were produced by coronary occlusion, they were all transmural in nature, while when produced by isoproterenol injection they were all scattered and small in size. This indicates that reproducible reliable conclusions only can be drawn from data obtained in animals with permanent occlusions.

Ca is believed to play an important role as a mediator for ischemia in isoproterenol induced myocardial infarction and this was described in detail by Blom and Davies in 1972. They showed that the amount of Ca in the myocardium increased and hydroxy-apatite crystals were formed in the mitochondria as a result of an uptake of Ca^{2+} ion. That the Ca pump plays a major part when myocardial necrosis is induced by isoproterenol was also confirmed by Lutmer et al. in 1971. It has been

postulated that the opening of the slow channels in the myocardial cell membrane allows this influx and accumulation of the Ca^{2+} ion into the ischemic and necrotic cell. In this type of necrosis it seems adequate to assume that pyrophosphate has a high binding affinity to the hydroxyapatite crystals formed. This would then explain the accumulation of pyrophosphate seen in rabbits when myocardial infarction was induced by isoproterenol injection.

In both the rats and the dogs an early uptake in necrotic tissue was found, although it occurred somewhat earlier in the rats. Already 4 hours after coronary artery ligation a distinct uptake was noted and the tissue radioactivity discriminated between infarcted and non infarcted areas. These results are in contrast to two other reports where Bruno et al. in 1976 found detectable uptake in necrotic lesions 7 hours post ligation, if it was a transient ligation, otherwise with a permanent ligation detectable uptake appeared 24 hours after ligation. Closest to our data was Buja et al. in 1975 who then reported uptake at 12 hours post permanent ligation and Martonffy et al. in 1976 who found no differences in tissue ratios at 18 and 48 hours after permanent ligation in dogs. When rats were examined histologically at 4 hours post surgery, cells in the process of dying were found. From these results and those of Schelbert and coworkers in 1976 it can be concluded that irreversibly damaged myocardial cells do take up considerable amounts of pyrophosphate. The early changes in the rat myocardial cells were described by Camilleri and coworkers in 1976 and the earliest changes found were microvascular in nature. Not until later an inflammatory cell invasion was registered. At this stage more cells were dying. The later in the process the animals were examined, the more increase occurred in the pyrophosphate accumulation. This would in the rats account for the increase in intensity that was found 24 hours and 48 hours post surgical induction of the infarct. Even in the 5 dogs sacrificed 8-9 hours after coronary artery ligation, histological examination showed early signs of infarction and this coincided with the uptake of pyrophosphate. A total disappearance of pyrophosphate accumulation on the 72 hour scintigrams in the rats examined in vivo and the intensity decreasing to 1 with the lesser tissue ratio at 72 hours. This in turn correlated with the total disappearance of all phagocytic cells in the infarcted areas and at that time only fibrotic tissue was left in the core of the infarct. Whether the time sequence found for pyrophosphate in

the rats is species specific is not known, but no similar results have been reported for any other animals such as rabbits, dogs, pigs or baboons. However none of these larger animals have the same fast metabolic turn-over as do the rats. No similar studies on guinea-pigs have been performed. The experiments with the rat showed a time course for the ^{99}Tcm pyrophosphate accumulation, that was similar and independent of the method for measuring the accumulation, but correlated with the removal of necrotic cells by phagocytes and the replacement by connective scar tissue. This observation strongly suggests and, maybe even supports the findings by Schelbert et al. in 1976, that the cells which take up pyrophosphate are irreversibly damaged and programmed to be replaced by connective tissue.

The radioactivity was also found to be highest in the infarcted areas throughout the whole series of the vivo examined hearts from rats and from biopsies from dogs. A concordance was found with the scintigraphic recordings in rats, where the highest ratios also were registered between infarcted areas and the border zone. Only in one rat was the activity higher in the border zone, which otherwise in both rats and dogs were found to have intermediate radioactivity values between the infarcted and the distal areas. Others (Adler et al. 1976) have also reported radioactive pyrophosphate content in the border zone. If pyrophosphate was to accumulate most in the border zone this area would have the highest radioactivity value, a results not supported by this study and not by Dewanjee et al. in 1976, Tofe et al. in 1976, Marcus et al. in 1976. Bruno et al in 1976 showed in dogs that infarction in excess of 1 % of the left ventricular mass was detected. The border zone around the infarcted areas was by Fabiani et al. in 1975 shown to be very small, decreasing in size during the first 48 hours after coronary ligation. After 48 hours the border zone had almost vanished and Fabiani and his coworkers found that the area was contracted and that it consisted of normal myocardial cells. These results supports our findings that pyrophosphate accumulates in necrotic cells. That it disappeared simultaneously with a formation of the fibrotic scar may depend on a wash-out mechanism maybe similar to the mechanism for the electrolyte wash-out from infarcted rat hearts as described by Polimeni et al in 1975.

The mechanism behind the pyrophosphate accumulation was not

studied, but several experimental reports have shown that some kind of residual blood flow is necessary to obtain a pyrophosphate accumulation. Walsh et al. did already in 1975 show this, when they found that peak pyrophosphate accumulation paralleled some residual perfusion measured with $^{13}\text{NH}_4^+$. Similar results were obtained in a study by Zaret et al. using a dual isotope technique with both pyrophosphate and a regional perfusion isotope. Martonffy et al. in 1976 also showed that transient ligation of an occluded artery resulted in a greater accumulation of pyrophosphate than did permanent ligation. Long and co-workers in 1980 showed that the pyrophosphate uptake was increased in reperfusion ischemia in dog-hearts as compared to permanent ischemia. As myocardial cells die progressively as a function of time surrounding the developing infarct for several hours, this is a population of damaged cells that still can recover (Marokko et al 1973). Agents sensitive to these changes may offer a mean to diagnose and quantify myocardial damages, and pyrophosphate seems to have some of these characteristics. Bruno et al. in 1976 and Marcus et al. in 1976 were both able to prove in dogs with ligation of the left anterior descending coronary artery, that the segmental myocardial perfusion was dominant and thus an important determinant of the $^{99}\text{Tc}^m$ pyrophosphate accumulation by necrotic myocardial segments. Similar results have been reported by Chwojinik et al in 1981, showing that an infarction resulting in a decreased segmental ejection fraction was compensated for by an increase in another segment where the perfusion was intact. The only dog where ^{201}Tl was used for measuring the perfusion pattern simultaneously with administered pyrophosphate also indicated that the necessity of having some perfusion present in order for a pyrophosphate accumulation to occur. Similar results were obtained by Horner et al. in 1980 in their isolated rabbit model. Buja et al in 1975 and Marcus et al in 1976 showed that if less perfusion is present a dough-nut type of accumulation appeared. This has also been observed in scintigrams obtained in clinical studies. It is possible that the presence of a rather undamaged myocardial perfusion accounted for a significant part of the pyrophosphate accumulation in the rabbits with isoproterenol induced myocardial infarction. These infarcts were pharmacologically and toxicologically induced and did presumably not alter the perfusion pattern.

The pyrophosphate saturation of the infarcted areas in the rats was not

believed to be due to a carrier overload. Injecting 0,168 mg pyrophosphate in each rat, and assuming that the carrier is distributed evenly throughout the extracellular space which was assumed to be 25% of the rat body weight, meant that 19 micromol/ml pyrophosphate was added to the extracellular space. This concentration of carrier was not considered to influence the result of the study.

SUMMARY

The animal experiments have in these different species shown that with $^{99}\text{Tc}^{\text{m}}$ pyrophosphate acutely infarcted areas can be detected and possibly within 4 hours after onset of the infarction. In our study the infarcted myocardium contained the largest amount of radioactive pyrophosphate when compared to the border zone or normal myocardium. The accumulation of pyrophosphate in necrotic myocardium and possibly also its elimination from these areas seem to parallel the histological changes at least in the rat. This may be species specific and it remains to be established to what extent this conclusions are applicable to an uptake of $^{99}\text{Tc}^{\text{m}}$ pyrophosphate by the human heart in the clinical setting of AMI.

CLINICAL STUDY

GENERAL CONSIDERATIONS

Malmö is a town with a population of about 250.000 inhabitants and is served by only one hospital with one medical emergency ward. This means that all patients with any suspicion of cardiac infarction apply for consultation at this hospital or will be admitted to the CCU of the hospital through its emergency room. This unique situation of one hospital has been used several times for population studies and epidemiological studies (Johansson 1966, Sternby 1968, Langeland 1970 and Rausing 1976). The present study has also taken advantage of the one city — one hospital situation as all patients included were admitted through the emergency room.

The criterias for admission to the CCU used were the following in combination or alone

- a) central retrosternal pain with a duration of more than 20 minutes and not relieved by nitroglycerine sublingually,
- b) repeated attacks of central chest pain each with duration of more than 15 minutes,
- c) pulmonary oedema or severe dyspnoe which could not be explained otherwise,
- d) electrocardiographic evidence of AMI,
- e) severe dysrhythmias not registered on previous examinations,
- f) shock without suspicion of being hypovolemic or anaphylactic in origin,
- g) syncopal attacks

These criterias are similar to those used by Hofvendahl in 1971, Helmer in 1973 and Lessem et al. in 1976. Due to a shortage of beds at the CCU an age limit of 70 was adhered to when necessary, for a admission to the CCU.

In the study 635 patients were included and this population will be defined later.

All patients admitted to the CCU were clinically examined on admission, and several times daily. The patients' ECG were continuously

monitored during the whole stay at the CCU and blood pressures as well as heart rate measured by specially trained nurses on irregular time intervals, but several times daily. After 3-5 days in the CCU the patients were moved to the after care ward, which is staffed by the same physicians and nurses as the CCU.

ROUTINE INVESTIGATIONS

Electrocardiography

Routine 12-lead ECG, including lead I, II, III, aVR, aVL, aVF, V₁-V₅ and V₇ were registered using a direct writing ink jet recorder-Mingograph (Siemens-Elema, Sweden). The 12-lead ECG was recorded on admission and each day during the patients' stay in the CCU and at least once more before being discharged from the hospital. If any complications occurred further ECG-recordings were made, ECG monitoring was also carried out during the investigations with radio-nuclides. ECG interpretations were done according to the Minnesota code.

Serum enzyme determinations

Serum enzyme were estimated on admission and at least three subsequent mornings, and in 296 patients, selected according to the work load of the CCU nurses, twice daily, once at 7 o'clock in the morning and once at 7 o'clock in the evening for the first 3 or 4 days in the CCU —TABLE VIII—. The enzymes routinely determined were serum

TABLE VIII

AGE	MALE	FEMALE
20 — 29	2	0
30 — 39	7	2
40 — 49	21	2
50 — 59	60	17
60 — 69	80	23
70 — 79	55	22
80 — 89	<u>2</u>	<u>3</u>
	227	69

Total number of patients with more than 1 ASAT examination on the three first days.

asparatateamino-transferase (S-ASAT), and serum alaninaminotransferase (S-ALAT), lactic dehydrogenase (S-LD) and when S-LD was elevated its isoenzymes were determined by electrophoresis. The analyses were performed at the departement of clinical chemistry at the Malmö General hospital and TABLE IX shows the reference values for the laboratory.

TABLE IX

S-ASAT (GOT)	ukat/1	< 0.7
S-ALAT (GPT)	ukat/1	< 0.7
S-LD (LDH)	ukat/1	< 8.0

Other investigations

Before discharge from the hospital a chest X-ray was taken, and the relative heart volume expressed as millilitres per square meter body surface area was calculated (Lysholm et al. 1934). The X-ray was performed at the department of radiology at the Malmö General hospital where the upper normal limit for men is 500 millilitres per square metre and for women 450 millilitres per square metre.

Follow up

All patients with verified myocardial infarction, according to WHO-criterias, were followed in a special clinic at three weeks, six weeks, three months, six months and one year following discharge from the ward. Then they were followed at yearly intervals if no complications were present. Otherwise the clinical controls were dependent on the nature of the complications. At each occasion a physical examination was carried out, a 12-lead ECG recording performed, and at the three months and one year control a chest X-ray was included to determine possible changes in the heart volume. Laboratory examinations consisted of S-cholesterol, S-triglycerides, hemoglobin, erythrocytes, sedimentation rate, a fasting blood sugar and a urinary screening test for glucose, proteins, blood, leukocytes and erythrocytes and were carried out at each visit to the clinic. In the post infarction clinic it was also decided if the patient should return to work full time, partially or retire.

Autopsy

All patients in the study dying at the department underwent a post mortem examination at the department of Pathology, Malmö General hospital. Those patients who died later outside the hospital were examined at the department of Forensic Medicine, University of Lund.

SCINTIGRAPHY PROCEDURES

All patients were examined as soon as possible after admission to the CCU with a variation of 2–336 hours with a mean of 39 ± 12 hours, following onset of symptoms. The scintigrams were recorded at bedside in the CCU with a mobile gamma camera — Porta camera I or II (General Electric, USA) Fig. 7. The two gamma cameras used in this

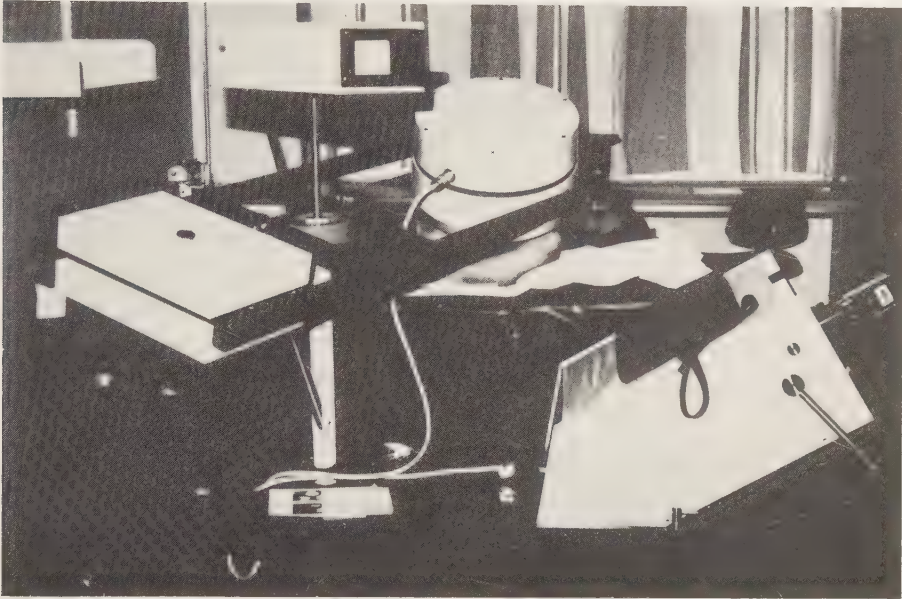


Fig. 7
The portacamera used in this study.

study have a gamma detector with a 35 cm diameter sodium iodide crystal and 19 photomultiplier tubes. The crystal is optically coupled to the photomultiplier tubes, which are arranged in a hexagonal way. Each phototube absorbs the secondary light, that photons produce within its own field of view. In the tubes the original number of electron is multiplied by a factor of 10^6 or 10^7 in order to produce an output pulse that is directly proportional to the total energy absorbed within the field of view of that specific phototube. The X and Y deflection signals are determined by a computing circuit and the event is then played on a oscilloscope as scintillation points — flickers and light. The Porta camera I only allows for one isotope study at the time, while the number II allows for dual isotope studies and has an intrinsic isotope

energy level adjuster. This was however not used in the present study. With both gamma cameras a standard low energy parallel-hole collimator was used and in each scintigrams 400.000 counts were collected. The pictures were recorded with a Polaroid camera and the camera intensity adjusted to the film sensitivity. In the majority of cases a three lens camera was used, allowing for three different grey-scales recording in each picture. The one with the best photographic quality was used for interpretation of the scintigrams.

The patients always received 10 mCi (350 mBq) $^{99}\text{Tc}^{\text{m}}$ pyrophosphate as the first of any radio isotope and if ^{201}Tl was given this was done 48 hours later in the amount of 1,5 mCi. All pyrophosphate scintigrams were obtained in the anterior-posterior (AP) and in the left anterior oblique (30 degree) position (LAO 30°), according to Bonte et al.in 1974, Parkey et al.in 1975, Lessem et al.in 1976, Holman et al.in 1977. Two identification markers of ^{57}Co were placed on the sternum with a constant distance of 10 cm, later allowing for an area measuring of the pyrophosphate accumulation. An AP scintigram was obtained immediately after radionuclide injection, and another one in LA 30° position immediately after the finishing of the first one. The interval between these two varied. These two blood pool images served as an anatomical orientation of the heart for later scintigrams as the heart contour is then well outlined (Lessem et al.1976). Scintigrams were registered every 15 minutes after injection, in both positions during the first 90 minutes after injection. All 5 minutes' scintigram and the 60 and 90 minutes' scintigram in both AP and LAO 30° positions were included in this study. All together 2540 scintigrams were included but 48 were not readable due to technical failures and 93 were not available for interpretation mainly because of previous use. This left 2399 to be interpreted not including the two 5 minutes' scintigrams. If these were included 3810 scintigrams had to be read.

The ^{201}Tl scintigrams were recorded 10 minutes after intravenous injection of 1,5 mCi ^{201}Tl (Philips-Duphar, the Netherlands) in AP, LAO 30° and the left lateral position as described by Wackers et al.in 1975, 1976, Walsh et al.in 1976.

EVALUATION OF SCINTIGRAM

In order to minimize the influence of variations the examinations were evaluated according to a standardized protocol. All AP and LAO 30° scintigrams were recorded 60 and 90 minutes after injection and were evaluated with regard to presence or absence of an uptake in the myocardial area. These scintigrams will in the future be referred to as AP 60, AP 90, LAO 60 and LAO 90. Each scintigram was compared to the initial 5 minutes blood pool scintigram, outlining the heart contours. An uptake on the 60 and 90 minute scintigram was only recorded as such if it was localized between the bone rib border and the sternum in any of the AP and LAO 30° scintigrams — Fig. 8 —. Absence of an uptake corresponded to an empty space between the two well defined structures. Two exceptions from these criterias were allowed. The first being a configuration of a new moon like shape among the ribs, and the second exception was if the uptake had the formation of an horseshoe and was overlapping two next to each other adjacent ribs, forming a bridge between the ribs —Fig 9—. Only one rib overlap was not accepted as a pyrophosphate uptake.

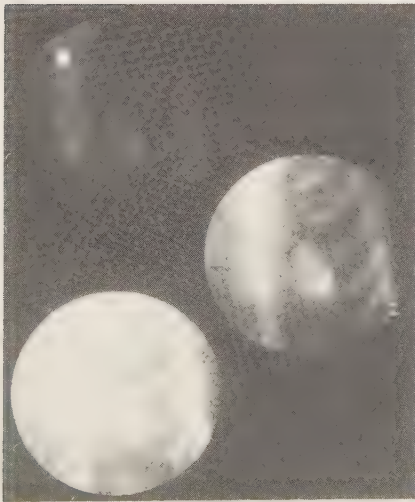


Fig. 8
The uptake area is illustrated with an uptake between the ribs and the sternum.



Fig. 9
An uptake in the form of a horse shoe and bridges between two ribs is shown.

Intensity of an uptake was graded visually according to a four step scale (0-3) where 0 represented absence of an uptake, 1 faint activity considered less than in the sternum, and 3 strong activity equally or more than the skeletal uptake seen in the sternum and in the ribs, and 2 being an intermediate activity. Diffuse activity was assigned to an uptake that spread all over the myocardium, whereas the localized uptake was an activity that was well defined to its borders, and did not occupy the whole blood pool area —Fig 10—. The localization of an uptake followed a schematic heart picture —Fig 11—, which was divided into different fields. These fields had been assigned numbers previously agreed upon by all investigators. The schematic heart pictures were based on drawings by Frank Netter (1969). Each observer was free to use as many localization numbers for every scintigram as he felt necessary. He could also change his localization of an accumulation from one view to another as well as the intensity could change. Before the evaluation started 10 different scintigrams were chosen to serve as an atlas to follow and where all five observers had agreed on the assignment of both intensity and localization. No question marks, pluses or minuses attached to the uptake and their intensity was allowed. The only comments allowed were if any scintigram or a whole series was judged as technically insufficient and uninterpretable or if a dough-nut pattern as described by Buja et al.in 1976, 1977 and Marcus et al.in 1976, occurred.

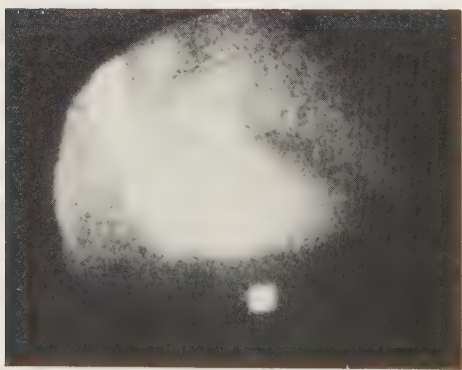


Fig. 10
An initial blood pool scintigram. A later pyrophosphate uptake should not cover the whole blood pool area.

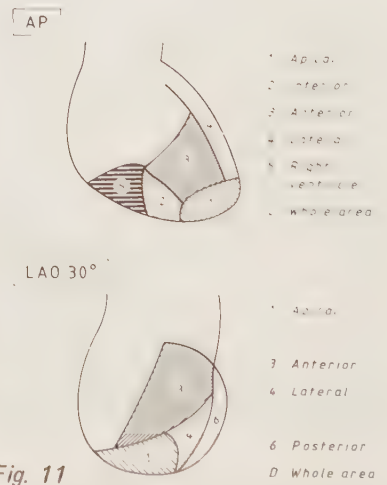


Fig. 11
A schematic picture of the heart used to localize an uptake.

OBSERVERS

All images were independently analyzed by five observers with different degree of experience in interpreting scintigrams. None had any knowledge of patient history, ECG or enzyme findings at the time of interpretation.

Observer number 1 (BN) and 5 (JT) both were attending physicians in nuclear medicine, but where BN had a few years more experience in evaluating scintigrams. Observer 2 (JL), 3 (SP), 4 (BWJ) were cardiologists but observer number 3 belonged to a department outside the hospital in another city. Observers 3 and 4 both are chiefs of cardiology, but observer 3 had very limited experience of scintigraphy when he started his evaluation. Observer number 4 had done some, but not much cardiac scintigrams reading, prior to this evaluation. Observer number 2 trained in cardiology, but had many years experience in evaluating myocardial scintigram and had spent a long period of time on nuclear cardiological research programs. Of all observers he was the most experienced in reading static myocardial scintigrams. The observers will hereafter be described as A-E —Table X—.

TABLE X

Observer	Initials	Specialty	Nuclear cardiological experience
A	BN	Nuclear medicine	Extensive
B	JL	Cardiologist	Extensive
C	SP	Cardiologist	Little
D	BWJ	Cardiologist	Moderate
E	JT	Nuclear medicine	Extensive

The table shows the experience in myocardial scintigram reading and specialty of the observers.

METHODS OF ANALYSES

As stated above the observers were instructed to record their interpretation for each image by rating the uptake in the previously described way. The sensitivity, specificity and predictive value of each of the levels was calculated for each individual observer using a two by two decision matrix, which relates to the presence or absence of AMI with a binary outcome if the imaging procedure was judged negative or positive (Turner 1978). The optimal or criterion level for each observer was decided to be that when a shift to a more lax criterion level caused a larger number in false positives as compared to the true positive fraction for the particular observer, and at the same time a shift to a higher level of criteria produced a greater decrease in true positives than in false positives. Receiver operating characteristic curves were plotted (Metz et al.), meaning that true positive was plotted against false positives for each observer.

For the clinical study an intensity level was defined for a positive scintigram. This was done for each individual observer, independent of the other.

The picture that yielded most information for each observer was determined. This was performed in all patients where all five observers had read all four scintigrams, 60 and 90 minutes after injection. Each patient was assigned a score which was the mean intensity value of all intensities multiplied by the number of equal intensities, this was called a weighted score which was then introduced into Fisher's discriminant analysis with matrix inversion and each patient was thereafter judged as having or not having an AMI. An intensity added score (IAS) for each patient and each position was also calculated.

The value of scintigraphy with either ^{99}Tcm pyrophosphate or ^{201}Tl separately or in combination with each other was assessed according to a method described by Wechio (1966) and again by Nosslin (1975) and Hall et al. (1976). The following equations were used:

$$1) \text{ sensitivity} = \frac{\text{true positive}}{\text{true positive} + \text{false negative}}$$

$$2) \text{ specificity} = \frac{\text{true negative}}{\text{true negative} + \text{false positive}}$$

According to the same equation the predictive value of a positive test can be estimated:

$$3) \text{ predictive value of a positive test} = \frac{\text{true positive}}{\text{true positive} + \text{false positive}}$$

The localization was compared for each of the observers with his own series of four scintigrams per patient, but also in comparison to the other observers' localizations and were plotted against each other. The localizations as determined by the observer was also plotted against the determined ECG-localization by the Minnesota code.

A special computer program using a PDP-11 computer (Digital Equipment, USA), was applied to the whole material when evaluated.

CRITERIAS OF ACUTE MYOCARDIAL INFARCTION

An investigational diagnosis of AMI was based on the presence of at least one of the following criterias (Hofvendahl 1971, Hellmers 1973, Erhardt 1974, Henning-Lundman 1975):

- 1) subjective symptoms with appearance of QRST-changes in the ECG, consisting of the appearance of a new pathological Q-wave, pathological R-wave progression or localized ST-T elevation followed by T-wave inversion in two or more of the leads persisting for more than 24 hours. If these changes were persistent the patients were assigned a diagnosis of transmural AMI (meaning an involvement of all the layers of the heart muscle wall). If however these changes were transient and returned to normal the diagnosis of subendocardial AMI was assigned to the patient.
- 2) subjective symptoms and two elevated S-ASAT values with peak value about 24 hours after onset of symptoms in combination with an S-ALAT lower than S-ASAT and with a peak about 36 hours following onset, of two evaluated S-LD values with a maximum about 72-90 hours following onset and an electrophoretic pattern dominated by rising isoenzyme I and/or II, or one elevated S-ASAT in combination with one elevated S-LD.
- 3) a combination of ECG changes and serum enzyme elevation as described above.
- 4) autopsy findings compatible with the diagnosis of myocardial necrosis and correlated in time with onset of symptoms.

CRITERIAS FOR SUSPECTED ACUTE MYOCARDIAL INFARCTION

The diagnosis of suspected AMI was assigned to patients with central chest pain with typical pain radiation, or pulmonary oedema or dysrhythmias and simultaneously showing ST-T changes indicative of infarction, but no Q-wave evolution and only one S-ASAT elevation or one S-LD elevation.

CRITERIAS OF ANGINA PECTORIS

Stable angina pectoris was defined as chest pain which was exercise correlated, relieved by sublingually administered nitroglycerine or rest, but without any signs of enzyme changes or new ECG changes.

The diagnosis of unstable angina pectoris (UAP) was divided into two subgroups differentiated by the enzyme response. Both groups had the following criterias in common:

- 1) angina of recent onset with progressive increase and severity of symptoms
- 2) angina at rest with recurrent episodes, lasting more than 20 minutes and poorly relieved by nitrates (Cairns et al.1976, Hultgren 1976),
- 3) transient ST-T wave changes during chest pain (Hultgren 1976, Duncan et al. 1976) often consisting of T-wave inversion and ST-T segment elevation or depression, normalized when the pain episode was over,
- 4) in the enzyme estimation two subgroups were defined:
 - a) one group where no serum enzyme elevation above normal values was allowed
 - b) one group where a 50% elevation of the enzyme values from base level was allowed without passing above, only reaching the normal reference value.

OTHER CRITERIAS USED

The ECG:s were read according to the Minnesota code. The localization of an infarction was judged as being anterior, anterolateral, septal, lateral, apical, apicolateral, anteroapical, right ventricular or any combination of these. The diagnosis of an old infarction was given to the patient who did not fulfill any of the above described criterias but who had a history of an old infarction with or without remaining QRST-changes to support the earlier diagnosis.

For the purpose of the present study the evaluation was based on the investigational criterias, which included more strict definitions than discharge diagnosis did. As can be seen from the TABLE XI a discrepancy between these two levels of criteria exists.

TABLE XI

Diagnose by investigational criterias	DIAGNOSE BY DISCHARGE CRITERIAS					
	AMI	SUSP AMI	ANGINA	SUSP ANGINA	OLD INFARCT	OTHER
T AMI	214	1	2	0	0	10
SE AMI	52	2	0	0	0	11
SUSP AMI	13	3	6	1	0	10
UNSTABLE ANGINA without enzyme	2	1	29	0	0	12
UNSTABLE ANGINA with enzymes	2	0	14	2	1	4
STABLE ANGINA	0	1	22	1	0	9
OLD INFARCT	9	1	3	0	7	32
OTHER	3	0	2	1	0	143
TOTAL	295	9	78	5	8	231
MISSING	9					

The discrepancy between investigational criterias and discharge criterias is demonstrated.

MEAN ASAT RATIO

An estimation using a mean ASAT issue served as index of the size of the necrosis. The ratio was calculated according to the following equation:

$$\text{meanquotient (Q)} = \frac{(\text{ASAT}_1 + N)}{n \text{ ASAT (xN)}} = \frac{N_1 \text{ASAT}}{(n \text{ASAT}) \times N}$$

where N = number of measurements done, and

n ASAT = the upper limit of the reference value for ASAT.

Everything above 1 was considered as a pathological quotient and will be related to peak S-ASAT values.

MATERIAL

Study population

635 patients with chest pain of the 2448 patients admitted to the CCU during 1975–1978 were included in the study and selected according to the daily capacity of the camera. No system of randomization was followed when including the patients. Of these, 487 patients were males with a mean-age of 61,3 years and 148 were females with a mean age of 64,4 years.

Based on the given criterias the discharge diagnoses are shown in TABLE XII —. Two hundred and ninety-five patients were discharged

TABLE XII

DIAGNOSIS	NUMBER OF PATIENTS
AMI without hypertension	246
AMI with hypertension	49
AMI suspicion	9
Status post AMI	9
Arteriosclerosis myocardi	8
Angina pectoris	79
Angina pectoris suspect	5
Pericarditis	5
Myocarditis	8
Arrhythmia cordis	27
Imcomp cordis	10
Oedema pulm	6
Cardiomyopathy	3
Heart neurosis	1
Valvular disorders	6
Rheumatic fever and cardiac disease	14
Liver disease	9
Gallbladder diseases	8
Pancreatitis	2
Embolia pulm	10
Bronchitis and pneumonia	9
Observatio	57
Malignant tumors	5
Hypertension	11
Diabetes	3
Alcoholism	2
Inborn errors of metabolism	3
Cerebrovascular disorder	3
Others non cardiac	20
No information	9
	635

The clinical discharge diagnoses in the patient population.

with a definite diagnosis of AMI, 9 had a suspected but not verified acute infarct, 9 had a history verifying an old infarction. Seventy-nine patients were discharged with a diagnosis of angina pectoris which was not divided in stable or unstable angina at discharge, but only later. Another 5 patients had suspected but not verified angina pectoris. Pericarditis was found in 5 patients, myocarditis in 8 and 10 showed clinical evidence of cardiac decompensation and 27 were discharged with a diagnosis of arrhythmias. Of the 635 patients, 57 (9,0%) were discharged without a definite cause being found for their illness, and in 61 the cause for hospitalization was found to be noncardiac. Sixty-two patients were discharged due to other cardiac disorders not of ischemic origin. In 9 patients information (except scintigram results, age and sex) were missing.

Onset

Three hundred and sixty patients (56,7%) had a single type onset of the illness, with a specific time given for being taken ill, while the remaining 275 had a multiple type onset of the disease with several attacks of e. g. chest pain, varying in severity before seeking medical advise.

Peak enzyme level

Two hundred and four patients (32,1%) had a S-ASAT maximum below 0,69 microkat/litre which is lower than the upper limits of the laboratory reference values. The remaining 431 patients had a S-ASAT maximum above this upper level, but only 34 patients showed a level above 10 microkat/litre as a S-ASAT maximum. The diagnoses of these patients are presented in TABLE XIII —.

TABLE XIII

S-ASAT MAXIMUM VALUES ABOVE 10,00 microkat/l

Diagnose by invest. criterias		Diagnose by discharge criterias	
AMI	21	AMI	
Unstable angina pectoris	1	Angina pectoris	
Old infarcts	2	Liver disease observation	
Other diagnoses	10	Arrhythmias	
		Embolia cerebri	
		Embolia pulm	
		Valvular disease	
		Diabetes mellitus	
		Matabloic disease	

Diagnoses of patients with S-ASAT maximum values above 10,00 microkat/l.

Complications during hospitalization

Of all patients 24 developed pulmonary oedema, 11 cardiogenic shock and 24 ventricular fibrillation. All together 120 patients showed ventricular extrasystolies. Of all patients only 13 developed atrial fibrillation.

Follow-up period

The follow-up period varied from 3 weeks to 5 years after discharge. Only 1 patient has been followed for a 5 year period.

Mortality

Thirty-two of the patients with AMI died during the hospitalization period, giving a mortality rate of 12,2%. Of these patients 10 (5 females and 5 males) died due to an irreversible ventricular fibrillation, while 8 died due to a cardiogenic shock and 14 of asystole. After discharge another 81 patients –26%– died of autopsy verified myocardial infarction during the follow-up period, the interval being 4 weeks to 2 years after the primary admission. Of these patients 63 had been discharged at the first hospitalization period with a diagnosis of AMI, while the remaining 18 had been discharged with other diagnoses –TABLE XIV–. Another 52 patients died after discharge, but of non cardiac diseases.

TABLE XIV

No of pat	Discharge diagnosis	Death diagnosis
63	AMI	AMI
18	Non AMI	AMI
52	Non cardiac	Non cardiac

This table shows the distribution of death causes after discharge.

INTEROBSERVER STUDY

INTRODUCTION

The result of several clinical investigations may vary when different observers evaluate the outcome of these investigations. This has been shown for ECG evaluation and for coronary arteriography and for different nuclear medicine procedures. Intraobserver variations have been reported for ^{201}Tl scintigraphy (Trobaugh et al. 1978, Atwood et al. 1981) and for ^{99}Tcm pyrophosphate scintigraphy (Cuaron et al. 1979, 1980). The aim of the present study was to investigate interobserver variations in a large clinical population, when the interpretations were performed blindly and to evaluate its impact on the clinical results, determining sensitivity, specificity, efficiency for the different observers. An attempt to evaluate which picture for each observer, and for the group as a whole, that yielded best agreement with clinical results was also made.

MATERIAL

Myocardial scintigrams from 635 patients were obtained in the previously described way. In 436 all four scintigrams were present for evaluation, and in the remaining 199 some were absent for each patient. The absent scintigrams had been used previously for educational purposes, some had been published, and could not be retrieved.

The five different observers have been described previously, and will in the future be labelled A—E.

METHODS

The number of scintigrams with the different intensities in the two described patient populations were added together and thus a mean intensity value for each observer was obtained. The outcome for each picture for each observer was also calculated.

A weighted score was obtained for each patient, being the sums of equalities in intensity multiplied by the mean intensity value. In addition an intensity added score (IAS) for each patient was also calculated, being the addition of the intensities present in the different pictures for all observers.

The weighted score varied from 0 – 60,00 but was divided into 13 subgroups of 4,99 units in each, and the intensity added score also varied from 0–60 but with 15 subgroups each being 4 units.

Statistical calculations were performed with Students t-test. Bayes theorem was applied as described earlier for sensitivity, specificity and predictive value calculations. Efficiency was calculated as the added sensitivity and specificity values divided by two. Receiver operating characteristic curves were plotted according to the previously referred method.

RESULTS

The analysis of the different observers' results showed a variation in the number of scintigrams read as positive. Analysing the results, it was found that the various observers showed a large discrepancy in the number of patients assigned to each intensity group in the four pictures –TABLE XV–XVIII. In none of the four pictures did an exact agreement occur for all observers. Most scintigrams judged as having an uptake were found in the AP 60 picture, and this was even for all five observers. In the LAO 90 picture the number of negatives was the highest. All observers were similar in finding fewer scintigrams with intensity three, and they all had more positive findings than negatives. They were also even in finding more scintigrams in all four pictures with intensity one, than with intensity two plus three, except for observer C in AP 60. This result became more pronounced in the two LAO pictures. Observer E differed significantly from the others ($p < 0,01$) in this respect as the scintigrams with intensity one was in great majority in all his pictures. There is a variation in the numbers in all four pictures. Scintigrams belonging to different patients were also judged differently by the five observers. One observer may even have judged an uptake as having an intensity grade three while another observer found an uptake grade one. The number of strong positives –grade three intensity– was equally distributed in all four positions except for observer C, whose number of strong positives decreased more from the 60 to the 90 minutes scintigrams than for the other four observers.

TABLE XV

Observer	AP 60 Intensity			
	0	1	2	3
A	113	173	132	18
B	92	173	136	35
C	70	154	195	17
D	142	158	122	14
E	130	229	72	5

This table shows the distribution of scintigram evaluation for each observer and each intensity group in AP 60.

TABLE XVI

Observer	AP 90 Intensity			
	0	1	2	3
A	164	174	79	19
B	100	174	127	35
C	101	226	103	6
D	146	150	127	13
E	193	194	46	3

This table summarizes the distribution of scintigram evaluation for each observer in each intensity group in AP 90.

TABLE XVII

Observer	LAO 60 Intensity			
	0	1	2	3
A	221	132	60	23
B	127	182	99	28
C	94	182	148	12
D	178	134	110	14
E	201	183	50	2

This table shows the distribution of scintigram evaluation for each intensity group in LAO 60.

TABLE XVIII

Observer	LAO 90 Intensity			
	0	1	2	3
A	240	124	48	24
B	136	181	88	31
C	198	220	72	6
D	176	138	110	12
E	264	133	36	3

This table summarizes the distribution of scintigram evaluation for each observer in each intensity group in LAO 90.

The distribution of the mean number of patients within each intensity group for all observers in the 436 patients – TABLE XIX. This table

TABLE XIX

Intensity	Observer				
	A	B	C	D	E
0	184	113	101	161	197
1	151	178	195	145	184
2	80	113	130	117	51
3	21	32	10	13	4

This table shows the mean number of patients within each intensity group, for each observer, in 436 patients.

shows that observer E found more negative scintigrams than the others but with the closest relation to observer A. Observer E's mean intensity value was 0,68, which was the lowest – TABLE XX – while observer B

TABLE XX

	Observer				
	A	B	C	D	E
Mean intensity value	0,86	1,14	0,96	1,11	0,68
$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	
SD	0,20	0,11	0,01	0,21	0,16

The mean intensity value for each observer. Observer E had the lowest mean intensity value.

showed the highest mean intensity value of 1,14. This observer also found more scintigrams with intensity three, 32, which was more than any other observer found. He actually found more scintigrams with intensity three than did the other two cardiologists, observer C and D together or the nuclear physicians observer A and E together. Observer E reported the lowest number of scintigrams with intensity three, only 4. Statistical significant difference existed between the observers' mean intensity values —TABLE XXI— and between observer E and B, a

TABLE XXI

Total mean value	A	B	C	D	E
A 0.86	×	*	NS	NS	NS
B 1.14	*	×	NS	* $p < 0.05$	** $p < 0.01$
C 1.11	NS	NS	×	NS	*
D 0.96	NS	*	NS	×	*
E 0.68	NS	**	*	*	×

The statistical significance of the differences between the total mean value for each observer is illustrated.

highly significant difference was observed ($p < 0,01$). The best similarity existed between observer A and E, and the least similarity between observer B and E. When the intensity values were compared for each picture, significant differences were obtained which in most cases reached the level $p < 0,001$. Between two of the cardiologists, observer C and D, the difference was obtained only for the AP 60 and LAO 60 pictures while no significant difference at all existed in the 90 minutes pictures —Fig 12—.

Weighted mean value																				
	A				B				C				D				E			
	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
A	a				*				***				NS				***			
	b				***				**				*				***			
	c				***				***				**						NS	
	d				***				***							***				***
B	a								NS				***				***			
	b								***				***				***			
	c								*				*						***	
	d								**				*							***
C	a												***				***			
	b												NS				***			
	c															***			***	
	d															NS				***
D	a																*			
	b																***			
	c																		***	
	d																			***
E	a																			
	b																			
	c																			
	d																			

a AP 60 b AP 90 c LAO 60 d LAO 90

Fig. 12

Statistical differences between the five observers evaluations in the different pictures are shown.

A close correlation for the total mean intensity value existed between different observers, and no significant variation was found –TABLE XXII–. The highest correlation was found between observer A and E with an r-value of 0,72, while between observer B and D the lowest correlation existed with an r-value of 0,55. The similarity was thus best between the observers A and E, while it was poorest between observers B and D.

None of the five observers attached the same intensity to the same number of scintigrams in all four pictures. For all observers a decrease was noted which was most pronounced in the 90 minute pictures. Wide range of intraobserver variation can therefore be assumed to operate in a material like this.

TABLE XXII

	A	B	C	D	E
A		0.69	0.66	0.66	0.72
B			0.62	0.55	0.62
C				0.56	0.67
D					0.59
E					

The correlation between the mean intensity values for each observer is shown. Best correlation exists between observer A and E, and the second best between observer A and B.

Exemplifying the spectrum of observer variations of the examined population, three patients were chosen —TABLE XXIII—XXV— and these examples illustrate the interobserver variations, but also the intraobserver variations. Observer E demonstrates a typical picture, by being one threshold below the other observers in intensity grading. The lowest intraobserver variation was found in observer A and B:s' interpretations, which was the case in the whole material. Observer B always showed least intraobserver variation.

TABLE XXIII

Observer	AP 60	AP 90	LAO 60	LAO 90
A	3	3	3	3
B	3	3	3	3
C	3	2	3	0
D	3	3	2	2
E	2	2	1	1

Thus table illustrates the interobserver variability in one patient who had an acute transmural infarction.

TABLE XXIV

Observer	AP 60	AP 90	LAO 60	LAO 90
A	3	3	3	3
B	3	3	3	3
C	3	2	2	2
D	0	0	0	0
E	2	2	2	3

This table illustrates the interobserver variability in a patient with non coronary heart diseases.

TABLE XXV

Observer	AP 60	AP 90	LAO 60	LAO 90
A	2	1	1	0
B	2	2	2	2
C	2	1	2	0
D	2	1	2	1
E	1	0	1	0

This table illustrates the interobserver variability in a patient with subendocardial AMI.

TABLE XXVI

Observer	Specificity		Predictive value	
	2	3	2	3
A	0,55	0,44	0,40	0,08
B	0,39	0,28	0,44	0,10
C	0,34	0,23	0,41	0,03
D	0,52	0,37	0,47	0,04
E	0,51	0,45	0,22	0,01

The interobserver variability is demonstrated for both specificity and predictive value of a positive test depending on the critical intensity grade.

Using Bayes Theorem, as described previously, the specificity and predictive value of a positive test was calculated for the mean value and for each picture observewise. Assuming that any positivity equaled an infarction the specificity and predictive value would than be 100% for all observers. Observer E was the one who had the lowest predictive value of a positive test, both for intensity 3—1,3% — and for intensity 2—22%—. The other observers had higher both predictive values of a positive test and specificity when an intensity level change occurred from 3 to 2 —TABLE XXVI—. Except for observer D, the other observers found highest predictive value of a positive test in AP 60, while their specificity was highest in LAO 90 —Fig 13—.

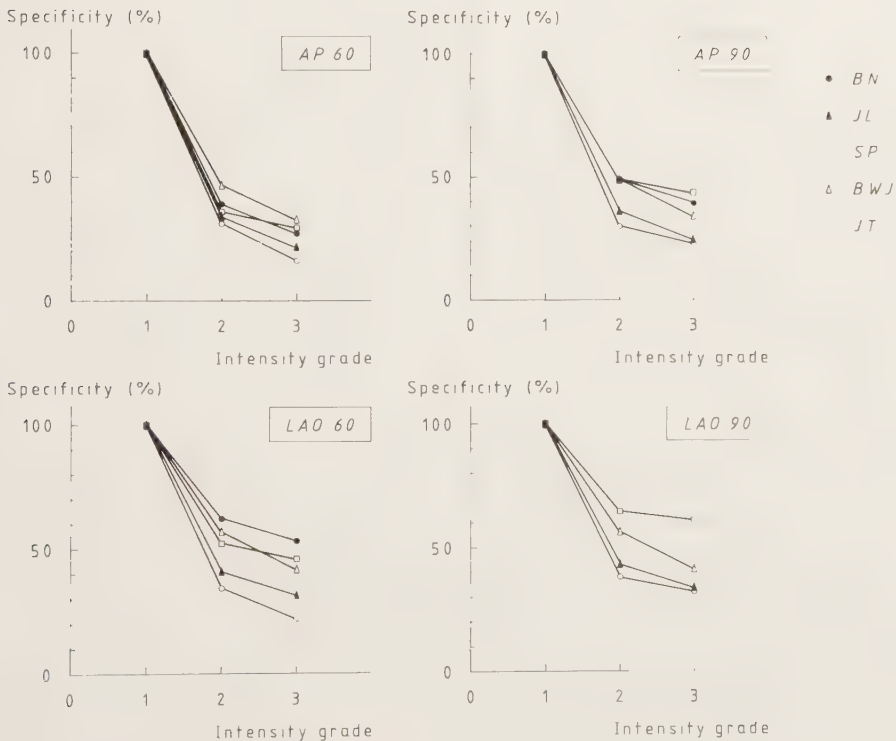


Fig. 13 a

Illustrates the outcome of the method for specificity in each one of the intensity intervals.

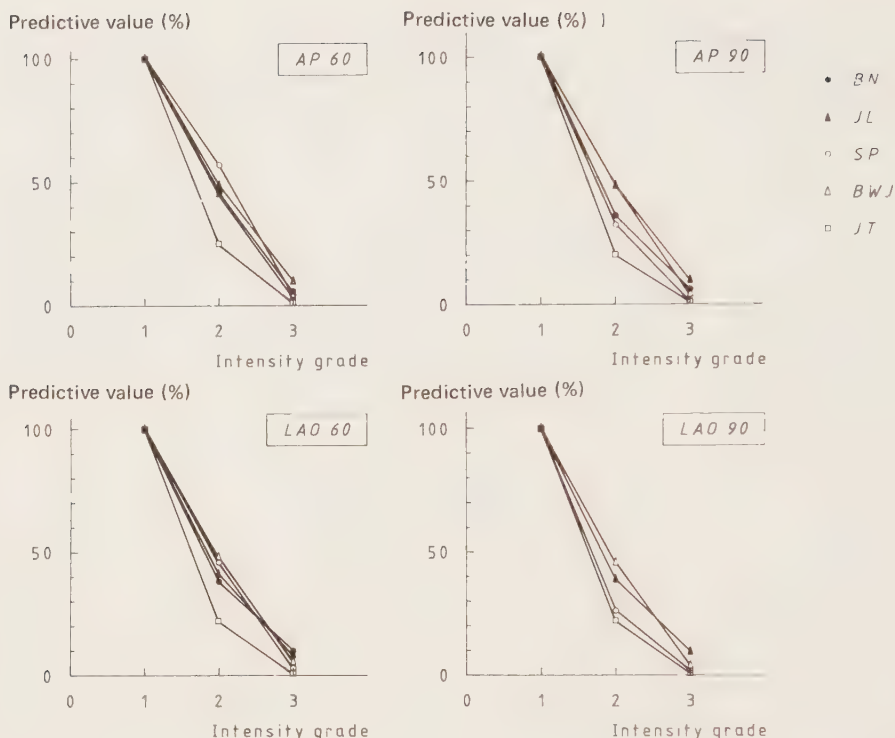


Fig. 13 b

Illustrates the outcome of the predictive value of the test in different intensity intervals for each of the observers.

When receiver operating characteristic curves were performed for all four pictures as well as for the total material, it was found that a rather small variation between the observers really existed, and that they were very similar when sensitivity and specificity was determined. Observer E tended to read more scintigrams as having the intensity one below the others, indicating that he had another threshold. However this difference disappeared again when specificity and sensitivity was calculated from the ROC curves. The optimal intensity grade for best specificity and sensitivity was somewhere in between one and two —Fig 14—, a finding independent of which picture was used, or if all pictures was added together —Fig. 15—. Accepting a lower intensity grade, the sensitivity

decreased while the specificity increased. The optimal intensity grade discriminating between an AMI and other diseases would vary between 1,4 to 1,6. As this intensity grade did not exist in the material and was not available to the interpreters, it was decided to use intensity grade two as a discriminatory point.

AMI - AP 60

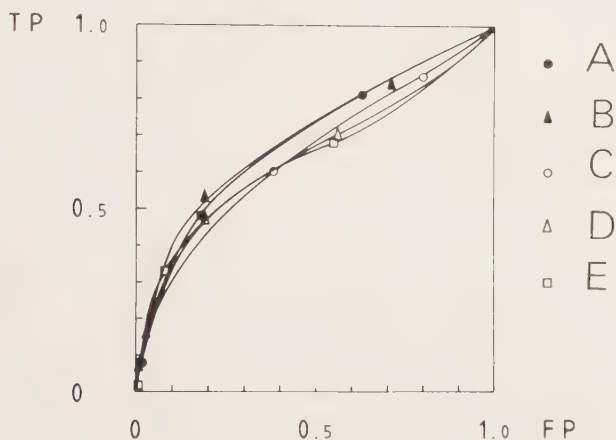


Fig. 14 a
ROC curves for AMI in AP 60.

AMI - AP 90

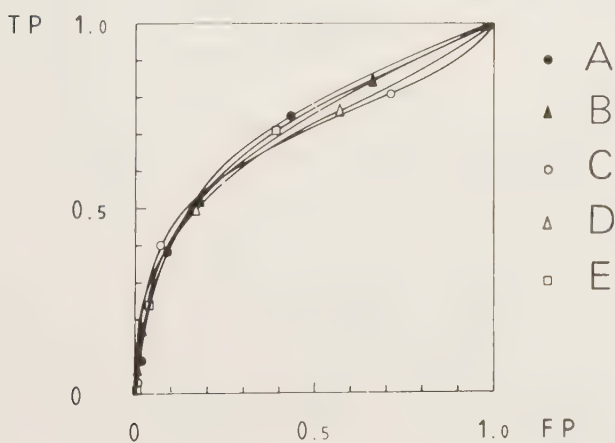


Fig. 14 b
ROC curves for AMI in AP 90.

AMI - LAO 60

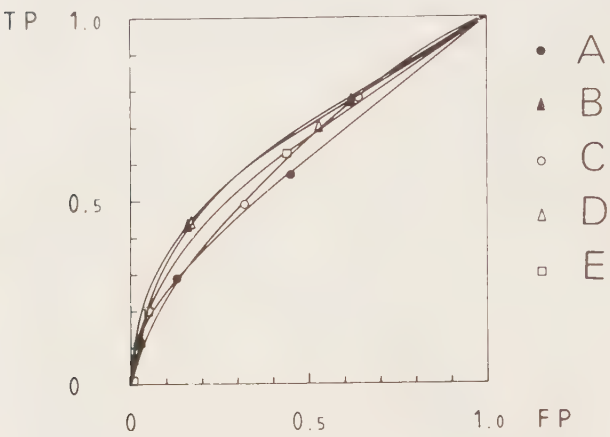


Fig. 14 c
ROC curves for AMI in LAO 60.

AMI - LAO 90

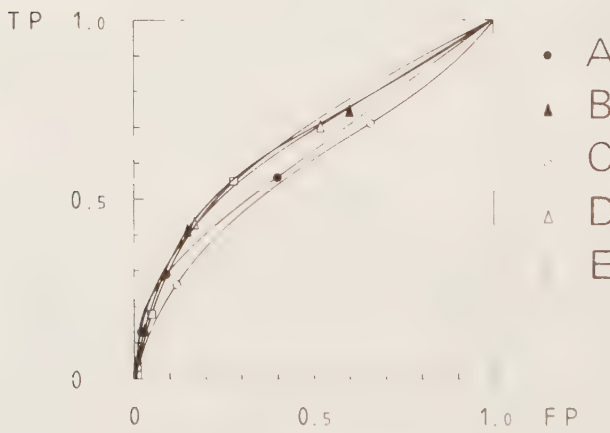


Fig. 14 d
ROC curves for AMI in LAO 90.

AMI - All projections

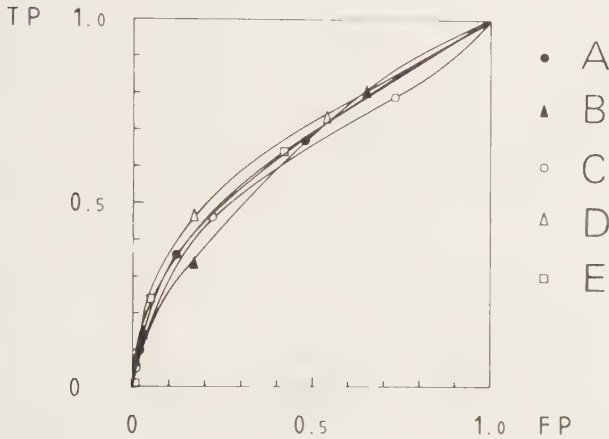


Fig. 15
ROC curves for the total population of AMI, in all projections.

The picture which for each observer yielded highest intensity values, was AP 60. The two AP pictures were the ones best discriminating between ischemic heart diseases and non ischemic heart diseases. The ROC curves confirmed these data, finding that the AP 60 picture gave the highest specificity and sensitivity for all observers.

The experience of the observer has been found to play an important role when judging myocardial scintigrams (Cuaron et al. 1980, Trobaugh et al. 1978). In the present study this fact is not so evident. The most experienced of the two nuclear physicians correlated rather well with the cardiologist most used to read myocardial scintigrams, with a correlation coefficient of the mean intensity value of 0,69. These two observers differed mainly in the number of negatives and in their predictive value of a positive test where the cardiologist showed slightly higher values. The other nuclear physician, observer E, more experienced in scan reading than the other cardiologists, had less highly intense scintigrams, while he showed a very large amount of negatives and faint uptakes, leading to the lowest specificity and the lowest predictive value of a positive test in the whole group. From observer E's ROC curve it is apparent that he placed his reading at a threshold lower, at

least for intensity grade two and three, than the other observers in the group. Observer C had virtually no experience in reading myocardial scintigrams, and he allocated the majority of scintigrams to the faint (1/3) and medium (2/3) intensity group. His discriminatory point for AMI against other diseases would therefore be closer to intensity one than two. If using grade two intensity as discriminatory factor four of the five observers presented a predictive value of a positive test varying between 44–46% while only one, observer E, had the extreme low value of 27%.

LOCALIZATION DETERMINATION

The observers also had to judge the localization of an uptake independent of the intensity, as has been described in the methodology, using the schematic heart pictures. In only 57 patients all observers were in total agreement concerning the localization of an uptake. The localization of the uptake, which in these patients was graded two or three, were anterior and apical or a combination thereof, and therefore rather large. All patients in this group had a transmural AMI.

The most common finding was a wide variation of localizations both inter- and intraobserver in the four pictures. Usually the same segments of the heart were seen in the two AP pictures, while different ones were seen in the two LAO pictures, a difference not explained by the different angles of the detector. The intensity attached to each segment varied also between the four different pictures, and again a wide variation existed both inter- and intraobserver. No consistent finding of a decrease or an increase in the number of segments seen existed with the four pictures and their time variation.

Observers B, C and D had more positive segments than observers A and E. B, C and D therefore had larger areas with an uptake than did A and E. The smallest variation of segments positive in all four pictures were found for observers B and D, while A and E were more constant within the two AP pictures and the two LAO pictures separately. Observer C exhibited the widest variation within the four pictures, but with no constant change pattern.

Diffuse uptake patterns were equally used by all observers, but observer B, C and D used it more often than two single segments. Observer B had most diffuse uptakes in the total population. He was also the observer with most experience in reading myocardial scintigrams with ^{99}Tc m-PYP.

A segment that seldom was judged as being positive was the right ventricle, which is in discrepancy with a recent report by Legrand et al. in 1981. None of the observers found more than 12 patients with a right ventricular uptake, and these were not the same for all observers. The variation of the localization of uptakes was for all observers independent of the final clinical diagnoses.

DISCUSSION

This study demonstrates clearly that there is a high degree interobserver difference in reading the scintigrams. We also found a high degree of intraobserver variability for the different pictures. Others have found the same for pyrophosphate cardiac examination (Cuaron et al. 1980, Lyons et al. 1980). These groups however could correlate the outcome of the study to the experience of the reader in evaluating the scintigrams, and especially myocardial scintigrams. Our study does not seem to support this assumption, as observer E being a nuclear physician was the one with least positive results. An agreement of the predictive value of a positive test between the other four observers is reported, and this is in concordance with what Trobaugh et al. has reported for TI scintigram readings, and similar to Atwood's results.

In judging the scintigraphic results no known bias were recorded. Observer E had his more strict level probably moved upwards leading to more negatives than the other observers and substantially less scintigrams with the highest intensity. This may well be due to a previous negative feeling about the method, thus a bias that was unknown before the investigation started. In contrast observer C had much less negatives and many more scintigrams read with a low intensity but with an uptake. This observer was also the least experienced of all observers. It is very probable that he had a positive feeling about the method, which gave the previous described results. Observer B had more scintigrams judged as having intensity three, than the other observers, and in this group almost all suffered from acute transmural myocardial infarction. This observer had by far the longest experience of interpreting myocardial scintigrams with a pyrophosphate technique. It may be speculated whether he had a bias or not, but no evidence for him being bias can be presented. His predictive value of a positive test did not surpass the others except for observer E's. Even if the material is large there was none who seemed to overdiagnose, but instead all observers seemed to underdiagnose. Cuaron et al. in 1980 claimed that this happened in their material too, but was at least in one case due to defect eye-glasses. No such external explanation can be found in this material. Instead it seems probable that this is a weakness of unbiased interpretation of any non mathematically well defined material which is to be evaluated. Legrand et al. in 1981 also found interobserver variability

between two observers, but solved the discrepancies with a third observer, who had only two choices. A similar approach was used for each of the four observers in Atwood's study, a so called dichotomous decision. Such an approach was not used in this study. It is not unlikely that the observers are intrinsically afraid of falling too far from what is believed to be the ideal interpretation. Such a mechanism may very well account for some of the discrepancies among the readers and their generally low specificity and predictive value of a positive test. The five observers correlated rather poorly with each other and also to previously published results (Turner 1978). It may even be concluded, from these data, that myocardial scintigraphy with $^{99}\text{Tc}^{\text{m}}$ stannous pyrophosphate in patients with suspicion of AMI is a very poor complementary instrument to other investigations. Such a conclusion based on these results is probably not accurate, and would be in discrepancy with most published reports. The previous conclusion would only be true if it is stipulated that all observers had to agree with each other on both intensity and localization in all four pictures. This may well prove difficult in such a large material as 635 patients with four pictures and four possibilities of intensity in each position. All observers should have given verdict whether a particular series of four scintigrams were considered positive or negative, but as this was not done a majority decision system had to be used. It has been suggested that when pyrophosphate myocardial scintigraphy is concerned one should accept one of the two concepts, either a floating scale where each observer finds his own optimal individual criteria level (Berman et al. 1977) or using a fixed criteria level where all observers are obliged to call an image positive or negative for AMI (Cuaron et al. 1980) and in the present study initially a fixed definition for each grade was used. This does not allow the observer to float along the scale. This may account for the threshold differences being present in the results, and it is well worth observing that the differences were more pronounced between the two highest intensity grades, than between any others. Especially for observer E this was true. From the results it is also apparent that the intensity grade two most probably was too widely defined, as the variation in this grade is the highest, which may be one explanation why the results in this study vary from hithero published data. Other studies on pyrophosphate (Walsh et al. 1977, 1978, Karunaratne et al. 1978) have reported that the method is very unspecific, which is supported by this study. Similar results

were found by Lyons et al. in 1980, who found that the localized pattern of radioactivity was 66% sensitive and 93% specific for AMI. However when its intensity was increased and the localization was still required to be well defined the sensitivity decreased to 28% in their material with a specificity of 99,8% for AMI. Others have found no difficulties in diagnosing AMI especially if these were transmural in nature. This is of course in discrepancy with the present study where less of the infarcts were accurately diagnosed independent of the observer. Together with other examinations the diagnostic activity towards this one entity was only somewhat increased. One reason for discrepancy between this study and others may be that in other studies the scintigrams were read by physicians familiar with the case history of the patient and not blindly as in the present study. In previously published reports from our own centre, the observer was usually aware of some of the clinical data concerning every patient, thus probably explaining the difference in result between this investigation and previously published reports from our own centre. Another more hilarious theory may be found in the reasons for uptake, where it is possible and at least can be speculated that different pathogenetical mechanism in the development of an AMI may or may not be responsible for the pyrophosphate accumulation, and that in this population fewer pyrophosphate uptakes reflect a different type of necrosis than has been the case in other studies. As the uptake of $^{99}\text{Tc}^{\text{m}}$ -PYP is both flow dependant, and dependant on the type of necrosis, similar discussions concerning the uptake pattern have been present in the literature (Wahner and Dewanjee 1981).

Another plausible reason for the discrepancy between the present study and previously published data, may be the used investigative technique. No background subtraction was used in our study, nor any enhancement procedures. At the time of the investigation no computer was available for corrections which means that all scintigrams read are the raw analog polaroid pictures. It is possible that the black and white contrasts was not optimal, and could have been improved with a computer. Therefore it seems plausible that with a computer technology the outcome of the present study may have been changed, but its feasibility as a quick bedside diagnostic mean can then be seriously questioned.

Results published where a combination of dynamic studies with pyrophosphate and a MUGA study have been used, confirm that more information about the infarcted myocardium is to be found (Corbet et al. 1981), that way. This difference in technique between that study and the present one may partly explain some of the discrepancies in results.

Observer agreement of about 80% have been found for a TI study (Trobaugh et al. 1978) but it may be that the readers were more used to assessing ^{201}Tl scintigrams of the myocardium than the observers in this study were to read pyrophosphate scintigrams. In another TI-study (Atwood et al. 1981) interobserver agreement was 75% for an abnormal study and 61% for a normal study. When they studied the interobserver variation for localizing a perfusion defect it was found to be 11–79%. One has to acknowledge the fact that potassium analog scintigraphy has been used for a substantially longer period of time and thus made people accustomed to visually interpret perfusion defects. It may also be dependant on that less background activity is disturbing the picture when the potassium analogues are used. For example no bone uptake exists. For coronary angiography an interobserver agreement of about 80% has also been reported (Derouen et al. 1977) However when a similar study was done with ECG recordings, the investigator conducting the study found as many interpretations of a single ECG tracing as he used interpreters. For this reason it is obvious that the value of the method can not be determined from an absolute blind reading without any knowledge of the patient at all, especially if a clinical diagnosis is sought for. Parkey et al. in 1976 read a much smaller material — 15 patients — in a similar way and found a better agreement between observers, but reached a sensitivity of 73%, which is about the same as this present study reports.

The over all agreement figure between observers could not be applied to any single individual study, but was in fact greater for all studies read as normal or with the highest intensity than for the two inbetween intensity grades. This is similar to findings in other studies, where interobserver variability has been thoroughly evaluated (Cuaron et al 1979, 1980). Among those with the highest intensity in all observers' reading, was one patient with no signs of cardiac disease. The anonymous reading makes it difficult to dismiss the suspicion of either a technical failure somewhere, a misplacement of scintigrams or a hitherto unknown uptake mechanism not associated with any intramitochondrial deposits.

It can of course be speculated that a hormone or changes in the Ca metabolism, as has been shown by Carr et al. in 1981, may be responsible for such a false positive patient. The particular patient was never reexamined. Interestingly enough he was examined with ^{201}Tl and had no perfusion defects that could be detected, which supports the hypothesis that the mechanism of uptake in this case was different from other known or believed uptake mechanisms.

When comparing the observers from various institutions it is of interest to see that the least experienced observer, coming from another cardiological clinic outside of the Malmö General Hospital, was the one who found less strong positives than the others and more with faint uptakes. This observer reached the predictive value similar to the others however. It can be assumed that he was more uncertain than the other four observers and therefore was tempted to use the faint and medium intensities more than the two extremes of the scale being negative scintigrams or the highest intensity grade three. Similar results were found by Trobaugh et al. in 1978 in their evaluation of Tl-scintigrams, where the observers belonging to the same institutions seemed to read scintigrams from their own institutions more favourably than those from other institutions.

Reasons for observer variation

Interobserver variability as such independent of the degree, has been described for several different reasons – TABLE XXVII –. Incomplete

TABLE XXVII

- 1) Incomplete knowledge of the method
- 2) Different levels of experience
- 3) Different visual perception
- 4) Communication between blinded observers
- 5) Observer bias
- 6) Adjusting criteria levels during study period
- 7) Not all observers blinded
- 8) Changes of visual perception over time

Reasons for inter observer variability.

knowledge of the study or technique could be one factor that would account for differences. This can not be said to be the case in this study, as all observers were well acquainted with the technique of examination, which has been previously well published. An atlas of typical scintigrams was agreed upon prior to embarking on the final interpretation study. Different degrees of experience of a certain technique or a study type, probably account for a larger amount of the variability and is probably a contributing factor in this study. Training in reading a specific type of data is of course an imperative factor in the variation, and seems to be confirmed by this and other reports (Cuaron et al. 1980, Smith 1967). It is also possible as has been shown, when this technique was applied on radiologists that their visual perception of a large field, does not include the whole area (Tuddenhem et al. 1961, Thomas et al. 1963). What bearing this has on the reading of myocardial scintigrams is not known, but it ought to play an important role, the larger the material is. Under the same heading falls the tiredness of the eye and mind to concentrate on the material for a longer period of time.

It is not known to the author, how long time each observer spent on the material or how many he read at a time, but it seems plausible to assume that only a certain amount could be concentrated on at the same evaluation occasion. Such a brake in interpreting may of course also change the level of perception probably back to a higher, when the continued reading is embarked upon. If communication between the observers takes place during the evaluation period, it may also influence the results in either direction. To the best of my knowledge such communication did not take place, and can therefore not account for the discrepancies between the observers.

Another factor that has to be taken in account is that the evaluation is made under conditions of uncertainty, and that the observer may have an intuition as to what the diagnosis of a patient as well as that of the scintigram may be. Only very obvious abnormalities will then be classified, as in this study, because background noise will disturb the observers evaluating capacity. This was reported (Lusted 1960) to be the case for interobserver variability in reading roentgenograms. Such a

mechanism may yield high sensitivity but a low specificity as Turner reports in 1971, and is then true for this study. By using a higher perception level, in this study grade three intensity, one would increase specificity but decrease sensitivity. It is the subtle differences by adjusting ones criterion level definition of the degree of abnormality in an image before classified positive, changing results of specificity and sensitivity will occur. Using ROC curves when evaluating an observer's result allow for working with a non fixed criteria level. Applying only sensitivity and specificity takes away their liberty in the clinical setting and assumes a constant criteria level over a long period of time, which is not likely to be the case otherwise. This is of course even more true if knowledge of the other clinical parameters e. g. in cardiac studies, values for the enzymes and ECG is added to the image interpretation, or if in e. g. roentgenographic examinations of the coronary arteries, the exercise test results are known in advance.

Such a working mechanism may not at all dismiss a method as less useful in detecting a disease, but by using the ROC curve for the observer in question this problem with a test might be avoided. In clinical practice and cardiological in particular it may well be advised to add information at the time of reading the scintigrams. These should of course first have been optimized as to background and signal to noise ratio. It is therefore also imperative that the equipment is kept at its best level of performance always. This may also eliminate some degree of interobserver variability.

The results presented in this study demonstrate that myocardial scintigraphy with $^{99}\text{Tc}^{\text{m}}$ -PYP is an examination that is not easy to interpret. It is questionable if one based on these data can claim it to be an investigation that can replace other methods. It seems more appropriate to suggest myocardial scintigraphy with pyrophosphate as an addition in the investigative tool arsenal instead of being a substitute.

SUMMARY

Interobserver variability was shown to exist to a substantial degree in the population of 436 patients where all five observers had all scintigrams available for interpretation. No differences occurred in the population of 199 patients where the observers not had all scintigrams available for interpretation. Significant differences existed in intensity evaluation, between all observers and this difference became more pronounced when each picture was studied. None of the observers reached a mean intensity value above 1,4–1,6 where the discriminatory point for the clinical evaluation of scintigrams should be. For clinical purposes and as 1,5 was not available to the interpreters, it was found that the intermediate intensity (2/3) would be appropriate to use for evaluation of the patient population. In this finding no interobserver variability was found. The presented interobserver variability was by far largest for the two end points of the scale (0 and 3) but less pronounced for the two inbetween intensity grades. The use of ROC curves demonstrates this smoothing effect clearly. Reasons for interobserver variability are presented. The preferred picture was the AP 60, where the predictive value of a positive test was highest. The specificity however was highest in LAO 90.

The interobserver variations found, yield doubts about the value of myocardial scintigraphy with $^{99}\text{Tc}^{\text{m}}$ -PYP performed with a simple technique as used in this study. These data do not allow themselves to definite conclusions as to the value of the method, as they are in discrepancy with many others where indeed more sophisticated techniques have been used. The previous experience in reading scintigraphy did not influence the result, but other factors and especially the total blinding of the observers may have played an important role. When used in a clinical surrounding the information obtained with myocardial scintigraphy may be of more value than these data suggest even if caution for its use seem to be well advised.

INDIVIDUAL OBSERVER EVALUATION

Each observer evaluated four pictures according to the principles described earlier. From these four pictures a synthesis had to be made answering the question whether a specific patient was positive or not for myocardial infarction. The sensitivity and specificity for each picture was derived and it was found that AP 60 and 90 yielded the highest specificity and sensitivity and therefore these two pictures were allowed to weigh more than the LAO 60 and 90.

From previous reports from our group, the intensity grading system has been the same as used in this study. Intensity grade zero and one have been considered negative and two and three considered positive. Using this criteria the outcome for the different observers could be calculated — TABLE XXVIII —. Observer E is the one with the lowest number of positives, while observer B is the one with the highest number of positives.

TABLE XXVIII

Observer	Positive	Negative
A	201	434
B	289	346
C	279	356
D	261	374
E	108	527

The table demonstrates the number of positives and negatives each observer found, when intensity grade two was discriminatory.

An investigation of a patient was defined as positive when three observers had two or more pictures with intensity two or more. If only two pictures had intensity two these had to be the AP 60 and 90. Using this criteria only 147 examinations were judged positive.

RESULT CLINICAL STUDY

MORTALITY AND SURVIVAL

Of all 635 patients 165 (26%) had died when the whole study period of four years was over, while the remaining 470 were still alive. Only 32 of the patients with AMI died at the ward, giving a mortality rate of 12,2%. It was found that 133 patients died later between four weeks and three years after discharge and of these 81 patients (60,9 %) had a verified AMI at post mortem examination.

SERUM ENSYME CHANGES

The distribution of peak S-ASAT values showed that the majority of patients had small S-ASAT increases. Seventy-three of the patients (11,5%) had peak S-ASAT levels above normal but they did not fulfill the diagnostic criterias for AMI. When the peak S-ASAT level exceeded 10 microkat/litre, 10 of these 73 patients were found and these all suffered from other diseases usually not associated with increases in the enzyme level. The majority of patients with myocardial infarction, 196 (66,4%) had a peak S-ASAT value below 4 microkat/litre, while 21 (7%) had a peak S-ASAT value above 10 microkat/litre. In 9 patients no information was available.

In 296 patients S-ASAT values were recorded both in the morning at 7 o'clock and in the evening at 7 o'clock. Of these 296 patients only 54 (18,2%) had a maximum value in the evening while the other 242 had a morning peak value. Only 31 (57,4%) of those with evening peak values had an AMI. In 11 patients with angina pectoris (6 with unstable angina and 5 with stable angina) a peak S-ASAT value was recorded in the evening. The relationship between the peak S-ASAT value and the calculated S-ASAT quotient for 619 patients is demonstrated in TABLE XXIX, and shows that the majority of patients had rather small infarcts. Of the remaining 16 patients there was no information in 9, and in 5 too scattered S-ASAT values had been taken for a calculation of the quotient.

TABLE XXIX

peak ASAT value	ASAT quotient							total
	≤0,99	≥1,00≤2,99	≥3,00 ≤4,99	≥5,00≤6,99	≥7,00≤8,99	≥9,00≤9,99	≥10,00	
≤ 0,69	203	1	0	0	0	0	0	204
0,69–0,99	44	18	0	0	0	0	0	62
1,00–1,99	16	87	0	0	0	0	1	104
2,00–2,99	0	68	3	0	0	0	0	71
3,00–3,99	0	36	4	2	0	0	0	42
4,00–4,99	0	18	12	0	0	0	0	30
5,00–5,99	0	2	16	2	0	0	0	20
6,00–6,99	0	6	12	4	0	0	0	22
7,00–7,99	0	1	9	4	0	1	0	15
8,00–8,99	0	0	6	2	2	0	0	10
9,00–9,99	0	0	0	1	3	0	0	4
≥10,00	6	0	3	11	6	0	9	35
total	269	237	62	26	11	1	10	619

The relationship between the peak ASAT value and the ASAT quotient is demonstrated. From this table it can be seen that most patients had low peak ASAT values, and small ASAT quotients. Only 347 patients had an ASAT quotient above 1, 355 patients showed pathological peak ASAT values.

TIME INTERVALS

The majority of the patients were examined with myocardial scintigraphy within 48 hours after onset of symptoms, and during the first day of their admission to the CCU – TABLE XXX and XXXI –. The discrepancy between these two intervals resulting in more patients being examined later after onset than later after admission, may be an expression of the patient delay or delay at the emergency room until admitted to the CCU.

TABLE XXX

Days	>0	>1	>2	>3	>4	>5	>6	>7	>8	>9	>10	>11	>12	
<1	≤2	≤3	≤4	≤5	≤6	≤7	≤8	≤9	≤10	≤11	≤12	≤13	≤14	>13
Patients														sum
98	135	107	70	47	32	28	20	13	17	16	4	5	5	38 635

This table illustrates the number of patients examined with myocardial scintigraphy in relation to the onset of symptoms. 53% were examined within 48 hours after onset.

TABLE XXXI

Days	> 0	> 1	> 2	> 3	> 4	> 5	> 6	> 7	> 8	> 9	> 10	> 11	> 12	> 13			
	< 1	≤ 2	≤ 3	≤ 4	≤ 5	≤ 6	≤ 7	≤ 8	≤ 9	≤ 10	≤ 11	≤ 12	≤ 13	≤ 14	≤ 15	> 14	
Patients																sum	
161	187	107	54	41	27		17	6	6	5	3	3	0	2	2	14	635

This table shows the interval between scintigraphy and admission This interval is somewhat shorter than between scintigraphy and onset of symptoms.

AMI DIAGNOSIS

For all scintigrams the intensity and the localization of an uptake was deterimed according to the criterias given earlier in AP 60 and AP 90 and LAO 60 and LAO 90. A typical grade three intensity pyrophosphate scintigram where all observers agreed and the patient had an AMI was a rare finding but is illustrated in Fig. 16.

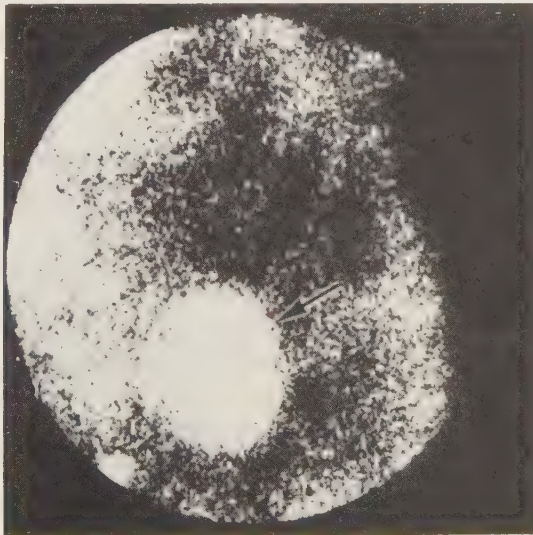


Fig. 16
A typical grade 3 intensity uptake, in a patient with AMI where all observers agreed.

The outcome for each observer was reported in TABLE XXVIII, and it is worth-while noting that none of the observers reached a number of positives that would total the patients with AMI. All observers however had patients judged as positive, but where the investigational diagnoses did not show an AMI — TABLE XXXII —. Observer E was the one who had least discrepancy with the clinical picture, but also the lowest number of positives with AMI.

TABLE XXXII

Observer	Positive with AMI	Without AMI
A	154	47
B	217	72
C	194	85
D	204	57
E	98	10

This table illustrates how many positives each observer found when intensity two had to be assigned to at least two scinitgrams, and then these had to be the AP pictures. The table also illustrates how many of these positives that did not have a clinically verified AMI.

All patients read were assigned a number according to how the intensities of their scintigrams were judged by the observers. This score was derived as a weighted score, where the steps between 0—1, 1—2 and 2—3 had been equalized. The formula used was: weighted score = $\frac{\text{equality}}{\text{mean intensity value}}$, which is described in a previous chapter. It can be assumed that for the different observers, the difference in intensity may vary but in view of the large population these differences should have lacked in importance as they ought to be equal in the material. Another score, the intensity added score (IAS) was an addition of the intensities, and another way to assign a number to each patient. Both these scores were applied to the patients.

The score reached at was applied to all 436 patients where all the observers had read all the scintigrams — Fig. 17 — and to the remaining 199 where not every scintigram was present for interpretation. Using this score system it can be seen that an overlap existed between patients with AMI, and patients with UAP and those with non coronary heart disease — Fig. 18 —.

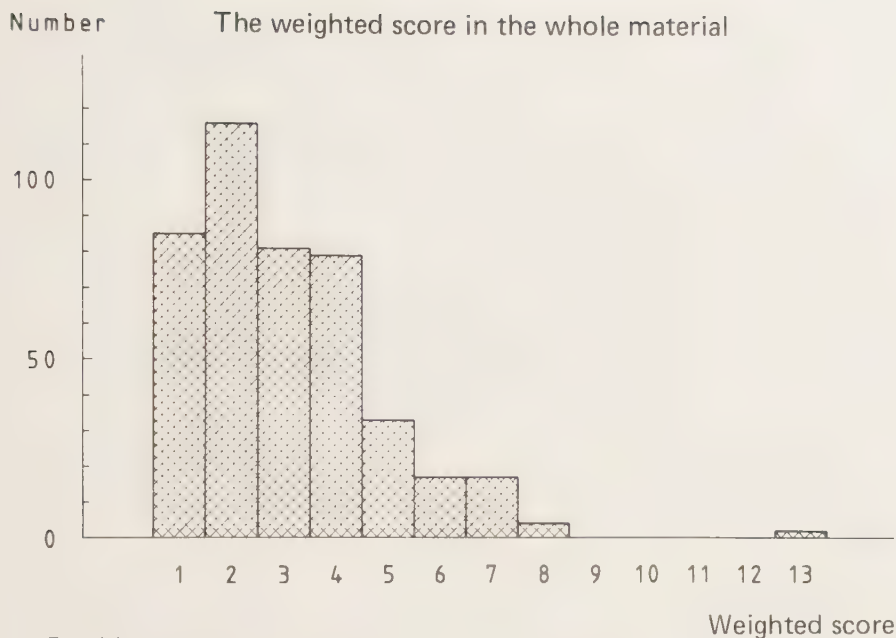


Fig. 17
The weighted score of the whole material is presented.

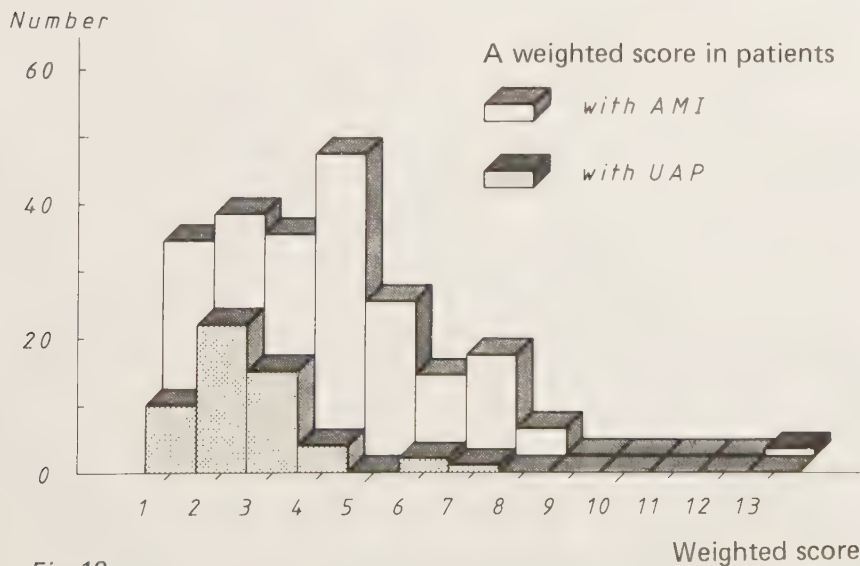


Fig. 18 a
This figure illustrates the overlap in weighted score between patients with AMI and UAP.

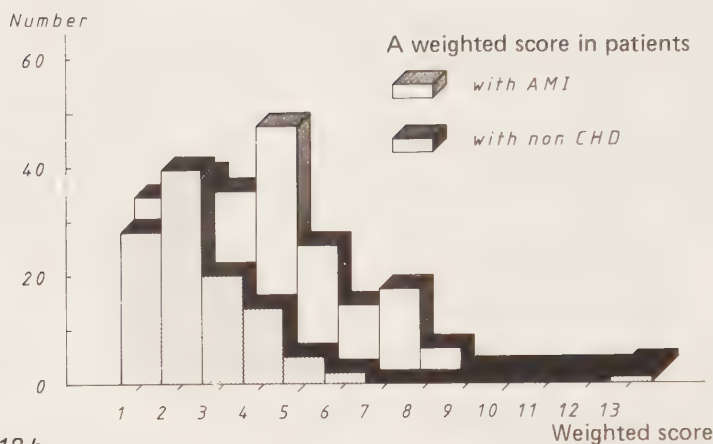


Fig. 18 b

This figure illustrates the overlap in weighted score between patients with AMI and non-CHD.

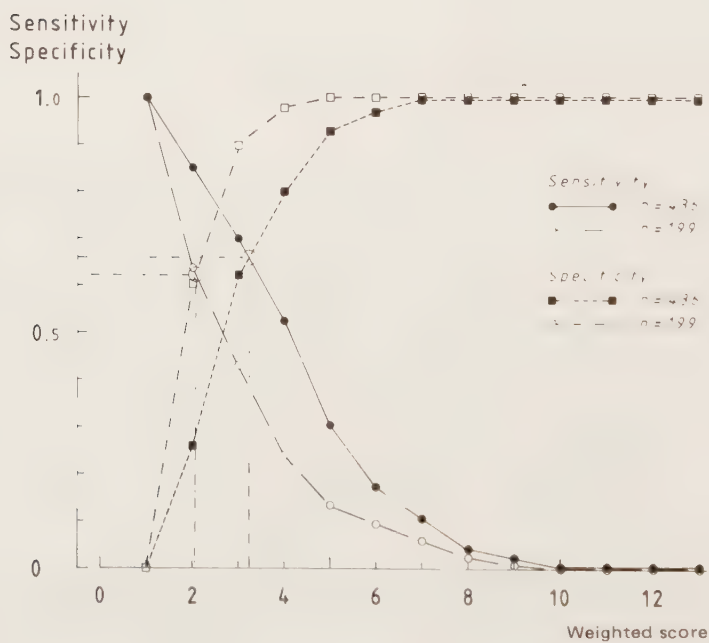


Fig. 19

Sensitivity and specificity is shown for the two populations. No difference between these two existed.

Sensitivity and specificity was calculated for the two groups separately and along a floating scale — Fig. 19 —. No differences between these two populations can be reported. It is noteworthy that the higher the specificity the lower was the sensitivity, and in the range where only definite grade three scintigrams were considered diagnostic for an AMI, a very high specificity was achieved but an extremely low sensitivity. The best diagnostic power in the method was to be found between intensity one and two, but at that intensity the discriminatory power is lost. The same was illustrated when efficiency was calculated — Fig. 20 —.

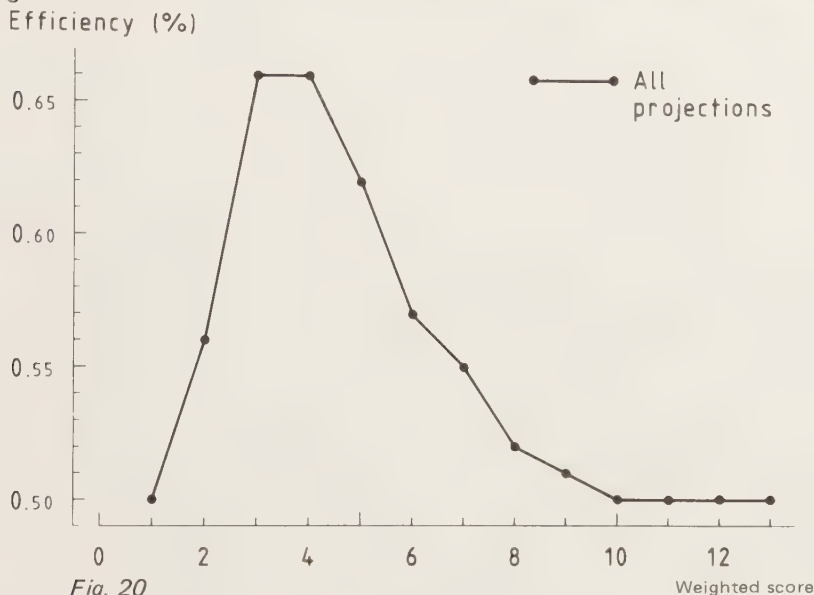


Fig. 20
The efficiency in the population was found to be 66%.

The predictive value of a positive test and a negative test was evaluated for AMI — Fig. 21 —. The method was more sensitive to negative tests than to positive.

When the four different pictures were evaluated separately, the highest sensitivity and specificity was found in AP 60 with an IAS of 7, while the lowest was found in LAO 90 — Fig. 22 —. The efficiency was also best for AP 60 — Fig. 23 —. No increased information over the single AP 60 picture was received when different pictures were combined.

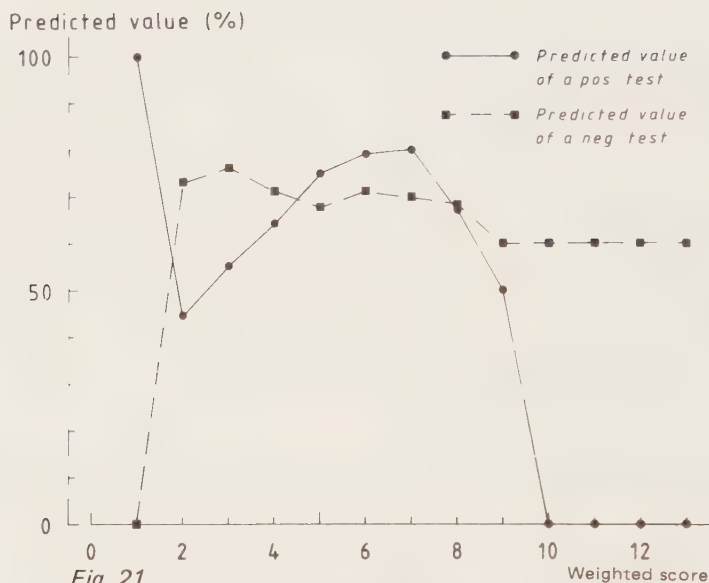


Fig. 21

The predictive value of a positive and negative test for AMI is illustrated. The highest predictive value of a positive test was 82%.

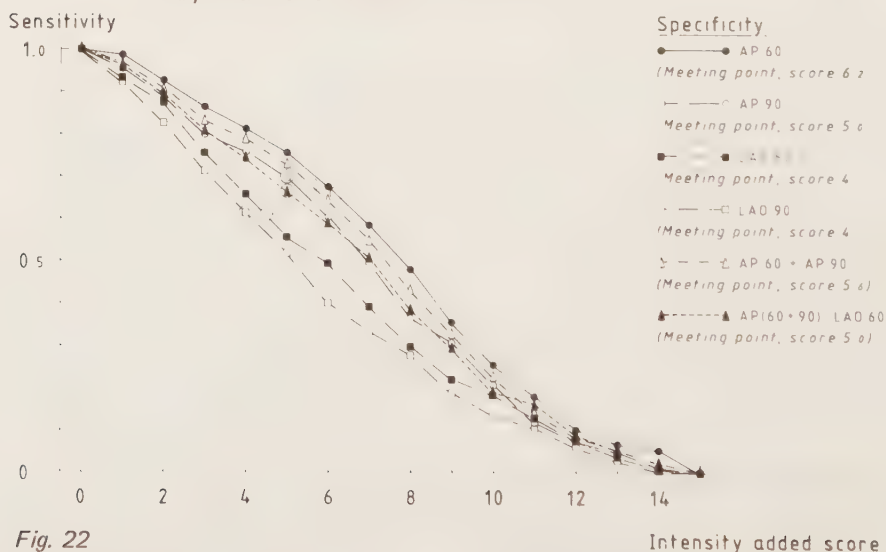


Fig. 22

The sensitivity and specificity for each projection and two combinations are demonstrated. The meeting points between sensitivity and

specificity range from 4–6,2 giving an intensity graded between 1–2. AP 60 is the position with most information.

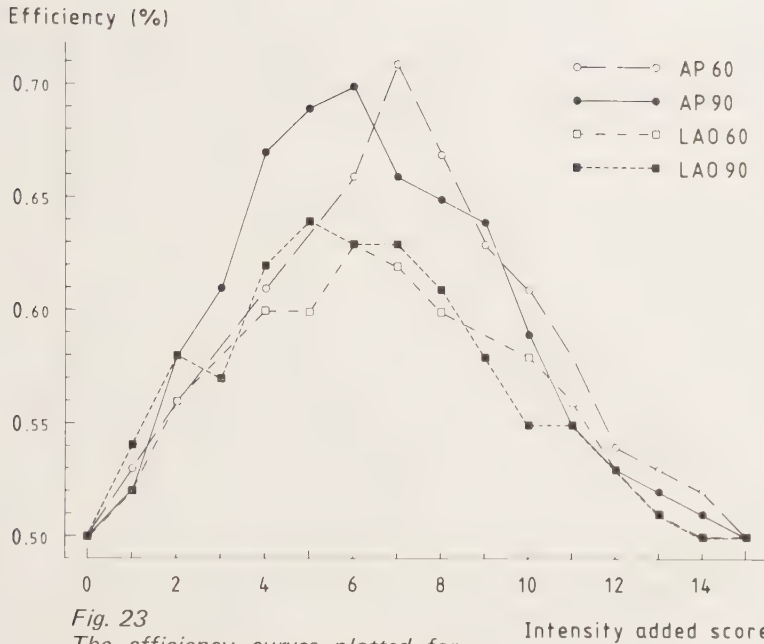


Fig. 23
The efficiency curves plotted for each projection. Note that AP 60 is the position where the efficiency is best.

If the score system was not used, but instead the requirement that the examination of a patient was positive only when three observers had intensity two in two or more positions, only 147 patients were found. Of these only 87 were patients with the clinical diagnosis of AMI. Using the criterias given, all observers found a substantial amount of false positive patients. The meaning of and reason for this will be discussed later.

One patient had a very well localized uptake and the intensity of this uptake was grade three by all five observers. This patient was discharged with the diagnosis of cerebral embolia. When retrospectively scrutinizing his medical history, all ECG taken, laboratory data and physical examinations, no signs of any ischemic heart diseases was found. However he was defibrillated due to a ventricular fibrillation of unknown origin a few days before examined. His cerebral embolia may well be a

residue of his ventricular fibrillation. Therefore it seems correct to label this scintigraphic examination false positive in this particular patient, being the only one with intensity three in all four pictures by all five observers and not having an AMI.

ISCHEMIC HEART DISEASE

The results from AMI diagnosing reported, showed that in this isolated entity myocardial scintigraphy was not too sensitive. In the literature it has been reported that the method is more valuable when the whole spectrum of ischemic heart diseases is concerned. In this study ischemic heart diseases as an entity was divided into AMI, stable angina and two types of UAP separated by an increase in the enzyme.

Only four scintigrams with intensity grade three were to be found in patients with the diagnosis UAP. Three of these four scintigrams belonged to the same patients, and two were read as grade three by observer B, and the third by observer A. In no patient with the diagnosis of UAP or stable angina pectoris were all four scintigrams judged as having the intensity grade three. In no patients did all the observers together find grade three or grade two intensity in all four scintigrams.

The patients with UAP were found in the low intensity group, and in AP 60 the sensitivity was higher for the low intensity grade — Fig. 24 — which was true for the efficiency which was highest when only a slight uptake was present. Most uptakes occurred in the AP 60 picture, which is in accordance with the findings for the group of patients with AMI. The peak of the sensitivity curve in this group was smaller and somewhat more well defined than in patients with AMI. As the peak sensitivity and peak efficiency is rather well defined in the group of patients suffering from UAP compared to those with AMI, a differential diagnosis between these two entities seems possible with this method even if the uptake mechanism for UAP is unknown.

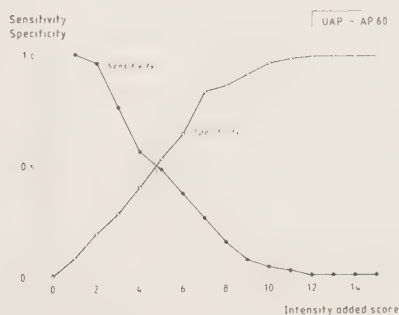


Fig. 24
The sensitivity and specificity for patients with UAP in AP 60.

ROC curves also confirm these findings, and show that the observers were more unanimous in their decisions of patients with UAP than in patients with AMI — Fig. 25 —. These curves also show a higher sensitivity and specificity for the low intensity grade as compared to patients with AMI.

UAP - AP 60

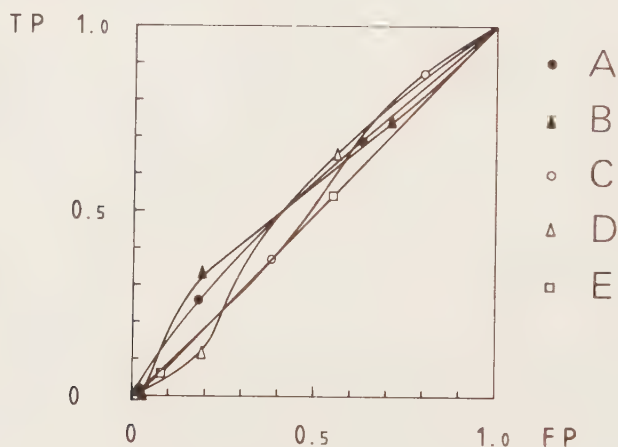


Fig. 25 a
ROC curves for UAP in AP 60.

UAP - AP 90

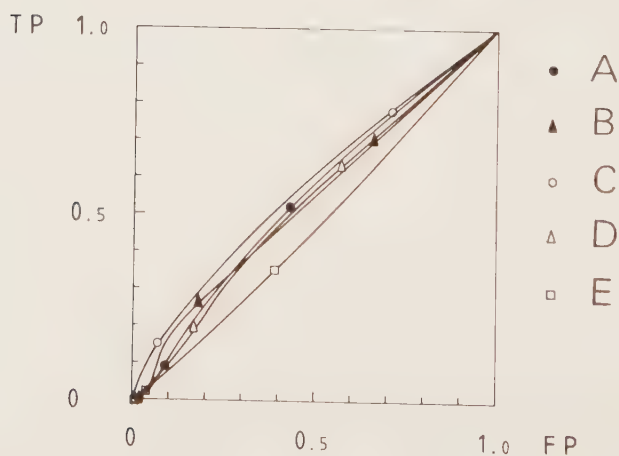


Fig. 25 b
ROC curves for UAP in AP 90.

UAP - LAO 60

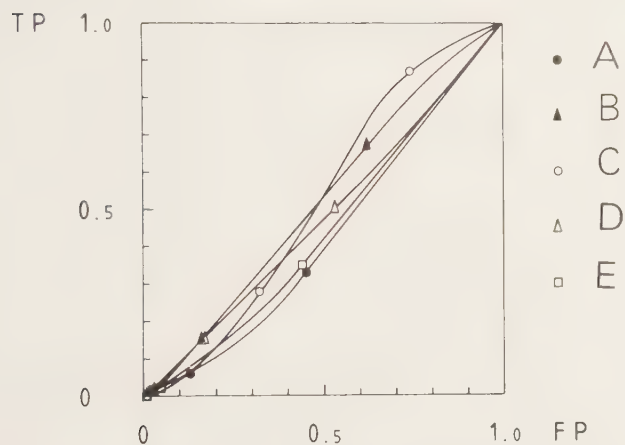


Fig. 25 c
ROC curves for UAP in LAO 60.

UAP - LAO 90

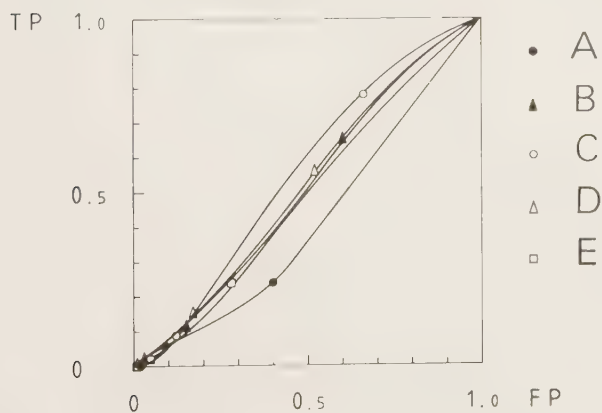


Fig. 25 d
ROC curves for UAP in LAO 90.

UAP - All projections

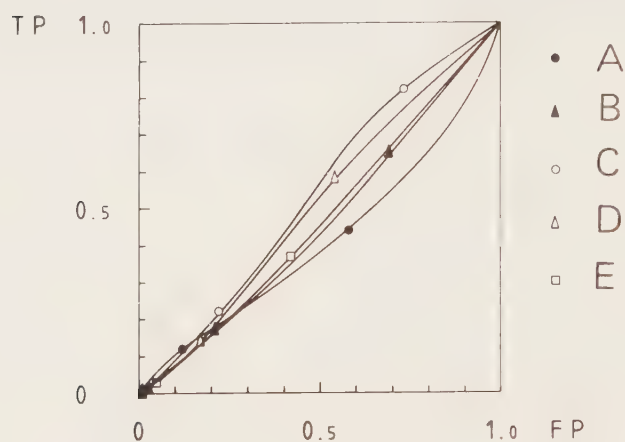


Fig. 25 e
ROC curves for UAP in all projec-
tions.

AMI - UAP - All projections

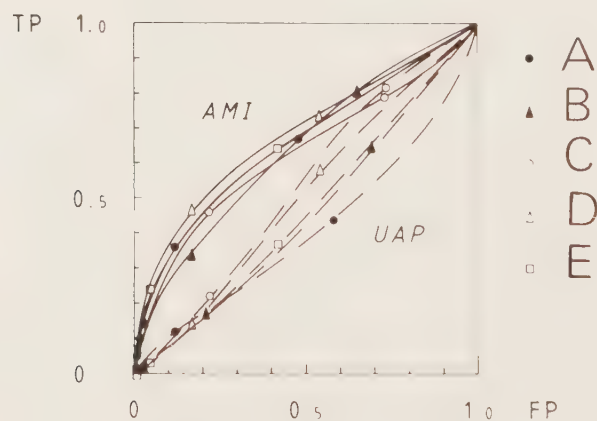


Fig. 25 f
The different ROC curves for AMI
and UAP are demonstrated. Only
for observer SP a small overlap
exists. The curves are discrimina-
tory for the two diseases.

For stable angina the ROC curves almost followed the line of identity for all observers in all projections and was rather similar to that of UAP.
 — Fig. 25 g —.

Stable angina - All projections

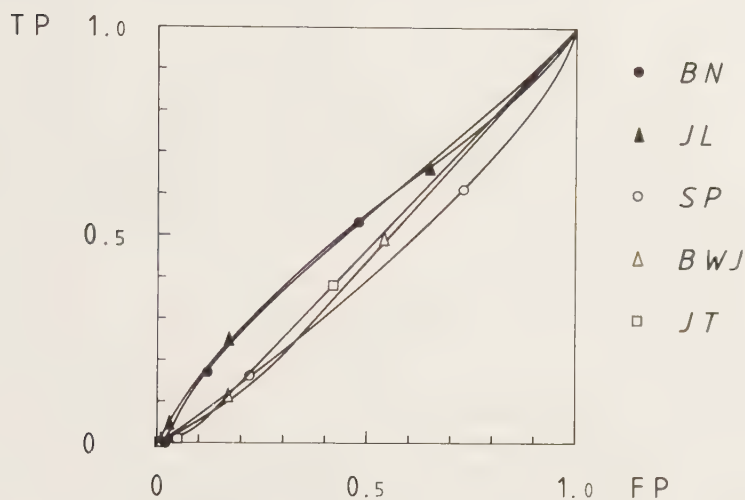


Fig. 25 g

ROC curves for stable angina pectoris in all projections.

EVALUATION OF THE METHOD

By calculating the mean value of the method, the natural course of myocardial scintigraphy with ^{99}Tcm -PYP would be described. The ideal situation would arise if discriminatory values were present for different diseases, at the same time discriminating normals from abnormals. Unfortunately this is not the case. There is a wide overlapping between the different diseases, and also with a normal population as can be seen from TABLE XXXIII. The mean values and the standard deviations are presented, and due to the big standard deviations, no significant differences can be detected. The difference between AMI and UAP is almost similar to the difference between AMI and non coronary heart disease. The mean value shows, that patients with AMI do have the highest mean values, $3,72 \pm 2,06$ while patients with UAP seem to have the second lowest mean values, $2,48 \pm 1,26$.

TABLE XXXIII

Diagnoses

AMI	$3,72 \pm 2,06$
susp AMI	$3,00 \pm 1,54$
UAP	$2,48 \pm 1,26$
stable angina	$2,40 \pm 1,36$
old AMI	$3,02 \pm 1,39$
non-CHD	$2,50 \pm 1,63$

This table shows the mean score value of ^{99}Tcm -PYP myocardial scintigraphy. A difference between AMI and UAP exists.

GROUPING OF OBSERVERS

Trying to improve the diagnostic power of the method it seemed logical to group the observers according to speciality. The uptake was still required to be graded with intensity two or more in the to AP pictures.

Observer A and E then found 73 patients whom they agreed on. Of these 8 had no AMI.

Observers B, C and D found 100 patients that they agreed on. Of these 100 patients 18 had no AMI. The difference between these two groups of observers was 4,2%, which was not statistically significant. It can therefore be concluded that grouping of observers in this population did not improve the diagnostic power of the method.

PROGNOSTIC VALUE

Of the patients judged as having uptakes graded with an intensity two in two or more of the pictures by three or more of the observers and a diffuse localization wide spread all over the cardiac contour only five developed an AMI. Therefore there was no significant uptake pattern in these five patients, that indicated a later progress of the ischemic heart disease.

Of those 81 patients dying after discharge of an AMI only 17 had uptakes judged as positive. This is only 21%. Another 20 patients had positive scintigrams for AMI, and these patients died later of non cardiac reasons, constituting 48,4% of the 52 patients dying after discharge of non cardiac diseases. There was no specific scintigraphic patterns in common for these patients dying, and thus the scintigrams had no prognostic value in this context.

Of the 32 patients with AMI dying during the hospitalization period, six (18,8%) had what was judged as a dough-nut pattern —Fig 26—, on the scintigram by all observers. They did however not agree on the intensity of the uptake, varying between two and three in the different pictures. On the localization of the dough-nut, there was also no definitive agreement, although all five had the uptake localized to the anterior and lateral wall, but two of the observers found no apical uptake while the other three did. These six patients dying were not the only ones having a dough-nut pattern, since one surviving patient with a severe complication of ventricular fibrillation also showed this pattern. It seems as if the dough-nut pattern is reserved for patients with complicated or fatal infarcts, and may be of prognostic value.

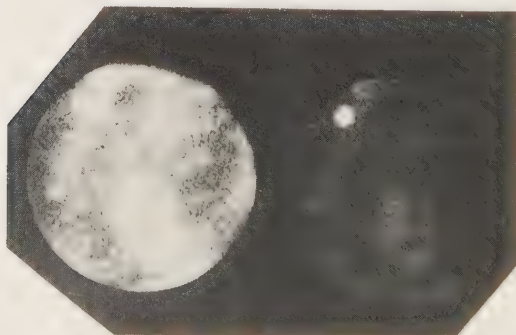


Fig. 26
A typical doughnut pattern of the PYP scintigram is shown.

CORRELATION BETWEEN INTENSITY AND PEAK S-ASAT VALUE

Several reports have claimed a correlation between MB-CPK release and the intensity of a pyrophosphate uptake. In this study MB-CPK was not performed, but instead S-ASAT. There was a wide spread scatter between the IAS and the peak S-ASAT values. Most peak S-ASAT values were as shown previously, found below 4 microkat/litre, and in this group the patients had IAS well below 40, which corresponds to an intensity grade between one and two. Even in the patients with peak S-ASAT values of more than 4 microkat/litre only three had an IAS of 60. No good correlation between size of the infarct judged with peak S-ASAT and the intensity existed. The trend however suggested that the majority of the infarcts in this population were rather small and may in turn have low intensity uptakes.

CORRELATION BETWEEN LOCALIZATION OF AN UPTAKE AND ECG CHANGES

The PYP uptakes were often found to cover larger areas of the myocardium than the ECG changes suggested. It was difficult to correlate the localization between these two diagnostic modalities as the localization of uptakes on scintigrams was very dispersed within the group of observers. As the PYP uptake is dependent not only on the necrosis but other factors, that does not influence the ECG, it is also difficult to correlate them to each other.

When comparing PYP uptake with autopsy data, a better correlation of areas affected was found.

TI STUDY

Of the 635 patients only a subset of 240 were examined with ^{201}Tl during the acute phase of the hospitalization period. This subset of patients did represent the majority of the diagnoses. Tl-scintigrams were read only by observer B, and the results are therefore only comparable to his PYP-readings.

Of the 163 patients who developed an AMI, this observer found 159 perfusion defects —Fig 27— and of the 39 with angina pectoris 9 showed a perfusion defect and 5 showed hypoperfused areas. In the group of 17 patients with old infarcts, all exhibited perfusion defects. No perfusion defects were found in the remaining 21 patients belonging to the group of patients with non coronary heart disease.



Fig. 27
An apical perfusion defect in one of the ^{201}Tl -scintigrams.

Of the 163 patients with an AMI and 159 perfusion defects, 157 showed PYP uptakes. One hundred and fifty-five showed pathological changes with both examinations, but two patients showed PYP uptakes who did not have a perfusion defect, and four patients had perfusion defects without PYP uptake. Of the patients with UAP, 24 showed PYP uptakes, and only 14 had a perfusion defect —TABLE XXXIV—. No correlation between the size of the perfusion defect and the PYP uptake was attempted. The PYP uptake in the patients with old myocardial infarction and non coronary heart disease were in three of the four pictures graded one by observer B. They would therefore have been classified as negative in previous parts of the study.

TABLE XXXIV

Diagnose	Number of patients	Present perfusion defects	PYP-uptake
AMI	163	159	157
UAP	39	14	24
old AMI	17	17	8
non-CHD	21	0	9

The table shows the data from the TI-scintigrams for observer B, correlated to his PYP interpretations.

DISCUSSION

DIAGNOSES AT DISCHARGE

Although this is no place for discussing the validity of the discharge criterias, it is noteworthy that if one uses the two different criterion levels for diagnoses, different results were obtained among the patients as seen from TABLE XI. It seems as if the investigational criterias used were somewhat preciser and more diversified than the WHO-criterias were, when ischemic heart diseases were concerned, but somewhat more lax when other disorders were concerned, a situation better describing the clinical problems. These criterias and the diagnoses are therefore thought to be more reliable. For the outcome of the scintigraphic results in this study it did however matter very little which of the criteria levels that was used, only small differences was seen which had no statistical value. For this reason the investigational criterias have been used all through the series.

As not all scintigrams were present for evaluation two populations existed. One population comprised 436 patients where all scintigrams were present for reading, and one population of 199 scintigrams where not all scintigrams were available for interpretation. These two populations are comparable, as can be seen from TABLE XXXV—, and the outcome of the scintigraphy was essentially the same in these both groups, which has previously been demonstrated. Therefore the discussion will mainly concern the group of 436 patients.

TABLE XXXV

Diagnoses	All scintigrams read	All scintigrams not available
T. AMI	132	95
SE. AMI	46	19
Susp AMI	25	7
UAP without enzymes	36	9
Stable angina	25	8
UAP with enzymes	18	5
Non-CHD	109	40
Old infarcts	42	10
Non information	3	6
Total	436	199

This table shows the two population studied according to the investigational criterias.

The results that patients with acute transmural myocardial infarction exhibit positive $^{99}\text{Tc}^{\text{m}}$ -PYP myocardial scintigrams confirm previous reports by Bonte et al. in 1973, 1974, Willerson et al. in 1974, 1975, McLaughlin et al. in 1976, Ennis et al. in 1975, Righetti et al. in 1977, Ahmad et al. in 1976, Parkey et al. in 1977, Bruno et al. in 1976, Okada et al. in 1976, 1977, Wackers et al. in 1976, Gould et al. in 1976. This study however showed lower safety in diagnosing AMI than does the above mentioned studies, and this is an apparent discrepancy which will be discussed later on in this chapter.

SIZING

Several attempts have been made trying to relate the intensity of a PYP uptake to the size of an infarct measured as enzyme release or depletion in histochemical studies. Marcus et al in 1976 could however not find any correlation at all between PYP scintigram intensity and the size of the infarct in dogs and similar results were obtained by Bruno et al. in 1976. Lessem, Clark and Menninger in 1979 showed no correlation between enzyme release and PYP uptake in dogs after permanent occlusion of the left anterior descending coronary artery (LAD). Other investigators however, like Botvinick et al. in 1975 and Parkey et al. in 1976, showed a very good correlation between the CPK release by infarcted myocardium and increased PYP activity. Similar Perez-Gonzales et al. in 1980 showed that PYP uptake reflects the size of the infarct and at the same time indicates a prognosis. In the present study no good correlation between the S-ASAT maximum value and the intensity was found. The same was true for the relative S-ASAT quotient used. As both intensity of an uptake, although it is time dependent, and S-ASAT values to some degree reflect the process of necrosis one would be justified to expect a correlation between these two parameters. It may have been that there would have existed a better correlation between CPK depletion and myocardial bound CPK units, and the intensity of the uptake. The procedure of determining CPK was at the time for the present study not routine laboratory procedure at the hospital and therefore not performed. However Wahner and Dewanjee in 1981 conclude that PYP uptake can not be used to quantitate the amount of infarcted tissue, but the area of uptake corresponds to the infarct size. As several studies have shown that the most intense uptake of Tc-PYP is located in the outer peripheral zone of the infarct

and that a close correlation exists with Ca deposits (Buja et al. 1979) it may well be that reflecting the size of the necrotic area is a task difficult for Tc-PYP.

OLD INFARCT

Ahmad et al. in 1976, 1977 and Klein et al. in 1976 both found that if a patient had a left ventricular aneurysm the PYP scintigram had also turned positive. Usually these patients have had previous acute transmural infarcts. However the activity was localized in the dyskinetic or akinetic areas of the left ventricle. Cowley et al. in 1976, and Wisneski et al. in 1976 both experienced that the low ejection fraction in combination with dyskinesia could lead to a PYP uptake. It has been speculated that the reason for the uptake is a calcification of the wall muscle. This may well be so, but in our study we found patients with old infarcts but no known left ventricular dysfunction exhibiting a PYP uptake, and where no difference in intensity compared to acute infarction were found. The reason for this secondary uptake remains obscure and it is not conceivable that only a scar tissue would yield an uptake, but more thinkable that the myocardium of these patients is constantly ischemic to some extent. These findings however decrease the use of PYP scintigraphy even in the acute stages.

FALSE NEGATIVES

Several patients with AMI both transmural and subendocardial, remained negative on the scan. Willerson et al. in 1975 and Berman et al. in 1975 have also reported negative scans and mainly in patients who later turned out to have had inferior infarcts either by ECG criterias, coronary angiography or autopsy data. Scrutinizing the literature so far, these seemed to be the only reports where transmural myocardial infarcts have shown no PYP uptake before the present study. However in patients with subendocardial infarction, Karunaratne et al. in 1976, Poliner et al. in 1976, and Parkey et al. in 1976 all found false negative results. Similar results were reported by Lessem in 1978. With other substances such as $^{99}\text{Tc}^{\text{m}}$ glucoheptonate, Holeman et al. in 1976 reported a higher incidence of false negative scintigrams in patients with transmural infarcts, compared to results with $^{99}\text{Tc}^{\text{m}}$ -PYP. Similar data was obtained by Lesch et al. in 1975. The number of false negatives increased remarkably in this study and was remarkably high when five

observers had to be in complete concordance when reading all four pictures. When this requirement was softened, and only one or two pictures or less observers were required to be equal, substantial amount of false negatives turned positives. A similar problem and a shift in observer requirement was seen by Cuaron et al.in 1980. Lyons et al.in 1980 also found similar results. The high amount of false negatives is also reflected in the low specificity and rather low predictive value of a positive test. One possible explanation for the high number of false negatives among infarctions could have been that these patients were scanned more than 7 days after onset when the possibility of positivity has been claimed to be decreased, (Willerson et al.1977), but only 35 with acute infarcts were scanned on day 7 or later, and therefore constitutes a less number than the false negatives do, but may still be a contributing factor. Another plausible explanation may be found in the fact that many of the patients were scanned within 48 hours post onset of symptoms, which according to the literature may be too early to obtain highly intense uptakes. From the S-ASAT data it is also apparent that a majority of the acute infarcts were small in size and they may have been too small to yield highly intense uptakes, but instead showed low intensity uptakes. Most published papers have used an intensity grading from 0–4, added with pluses and minuses to every grade. This investigation used the grades 0–3, and found that intensity grade 2 was to widely defined, which may account for differances with the literature. It is possible that our grade 2 may equal grade 2–, 3– and 3 in other reports, and that our imaginary grade 1,5 equals 2– and 2 which in most cases has been considered positive for AMI. The reasons for discrepancy with the literature anf the present study are summarized in TABLE XXXVI.

TABLE XXXVI

- 1) Scanned earlier in progress of disease
 - 2) Large number of small infarcts
 - 3) No computer manipulation with analogue Polaroid pictures
 - 4) Absolute blinding of observers
 - 5) To few intensity grades
- Reasons for discrepancy with literature.

The scanning time after injection has been kept constant, and should therefore not influence the number of positives or negatives. However in patients with extreme skeletal uptake it may be possible that these

uptakes interfered with the observer, and yielded a false negative. It is also possible that the technology available with no computer may have influenced the results. Many reports have shown that background subtraction or other manipulations with PYP scintigrams may indeed change the outcome and increase both sensitivity and specificity. The diagnostic value of either one of these two may however in the entity of AMI according to Galen et al. be equal. If one increases the sensitivity a lot of patients may be overdiagnosed, and on the other hand if one increases specificity a lot of patients may be missed. When Galen et al. compared ECG to enzymes they found that in the context of AMI equality of the two parameters was desirable. Concerning AMI one does of course clinically not want too many patients missed, and on the other hand not too many patients diagnosed as false positives. It seems therefore rational to equalize the importance of these two parameters.

The absolute blinding of both observers and scintigrams could also have attributed to the poorer result of this study as compared to others. No report with such a large material has hitherto been published where all data was blinded. In smaller materials Parkery and coworkers in 1977, have used an absolute blind technique. Legrand et al. in 1981 used a third observer to solve any conflicts between two other blind observers. In this study a majority decision was used.

It is also remarkable that during the last two-three years the number of publications on static myocardial imaging with ^{99}Tcm -PYP has decreased substantially and this may in turn indicate that several centres encountered too many false negatives and false positives but did not report this. Instead the method has been criticized and not used. Another probability is that other centres have very high reproducibility in reading the scintigrams, and feel very confident when diagnosing AMI with ^{99}Tcm -PYP. Our results do indicate that the use of myocardial scintigraphy with ^{99}Tcm -PYP is warranted as an additional information and diagnostic tool in selective patients.

Another factor may be that a false negative scan may be an indication of a long term poor prognosis, as it may signal a deficient collateral coronary circulation but no proof for such a possible mechanism can be presented by this investigation. From other studies by Perez-Gonzales in 1980 it is apparent that poor perfusion may yield very low intense uptake. Results by Long et al. in 1980 showed that there is a difference in PYP uptake when occlusion necrosis is present compared

to when reperfusion necrosis is present. This group showed that scans obtained with ^{99}Tc m-PYP were positive in only one of six dogs with sustained coronary occlusion when the isotope was injected 4 1/2 hours after sustained coronary occlusion. In animals with reperfusion necrosis twelve of thirteen had a positive test, and the isotope was injected 3 1/2 hours after reperfusion. These data again support previous results by Zaret et al. in 1976 where they found the highest concentration ratio of Tc-PYP between damaged and normal myocardium occurring when local blood flow was 20–40% of normal. From these animal results it may be possible to draw the conclusion that different types of necroses exist, and that different necroses yield different uptake patterns. It is also apparent that from animal experiment data it can be concluded that false negatives may appear in transmural myocardial infarctions, which are due to permanent occlusions, while false negatives may be more rare in patients with perfusion necroses. If coronary artery spasm is present it is then possible that less false negatives occur, while if coronary artery thrombosis is present more false negative results will occur, especially if the patients are scanned early in the course of an infarction. Later on when thrombolysis may have occurred, it is then plausible to suggest that the scans with ^{99}Tc m-PYP may turn positive.

FALSE POSITIVES

In this study patients with UAP were found to yield positive PYP scintigrams. Similar findings have been reported by Willerson et al in 1975, Parkey et al in 1977, Okada et al in 1976, Berman et al in 1976, Lessem et al in 1976, Ahmed et al in 1977, Gould et al in 1976, Karunaratne et al in 1976. The appearance of the PYP uptake in this group of patients significantly differed from the one seen in transmural AMI, in so far that the uptake was of lower intensity and diffusely spread. Lyons et al. in 1980 reported very similar results, but with localized uptakes of diffuse intensity. In patients with UAP, the uptakes reported are usually more wide spread, covering the whole myocardial area and of less intensity. Similar results are reported in this study, even if patients with UAP are to be found in all intensity groups except the highest. None of the observers found any patients with UAP with an uptake intensity graded three. Generally a wide spread localization was also noticed, confirming the localization pattern of other

investigations. Abdullah et al in 1976 found that PYP scintigraphy was an accurate and sensitive method in diagnosing UAP, Considering that the mean observer sensitivity for UAP of this study was 98,0%, it confirms the results of the Abdullah-group. The mean predictive value of a positive test for UAP was 13,5% which shows that for deciding if an uptake is dependant of UAP or not the accuracy falls considerably. The Abdullah group in 1976 also found that PYP scintigraphy in a group of patients with UAP did not yield any prognostic value as to what was going to happen to the patient and furthermore was of no importance for short or long term prognoses A positive scintigram did not mean that the patient was going to develop an AMI, and did not reflect the severity of the disease. None of the patients in their material died, and the same was true for our patients with UAP. Our result indicated neither any prognostic value of the PYP scintigram for the future outcome in these patients, nor can it predict a future AMI. No difference in these results were seen in the group of patients allowed an increase of 50% from starting levels of the enzyme. Donski et al. in 1976, Ahmad et al. in 1977, Gould et al. in 1976, Willerson et al. in 1975 and Abdullah et al. in 1976 all speculated whether the positivity of the scintigram in patients with UAP indicates that small and may be scattered myocardial necroses otherwise undetected are present and detected. No evidence neither by Cairns in 1976, Hultgren in 1976, Duncan et al. in 1976, Hsia-Teng in 1976 have been presented favouring the theory that UAP is an expression of small necroses in the myocardium. However Baroldi did in 1975 describe several kinds of pathological myocardial necroses where one type could well reflect angina pectoris. Long et al. in 1980 also described two different types of myocardial necroses, where maybe reperfusion necroses may well simulate the situation of general ischemia due to angina pectoris. When isolated heart preparations from rabbits were studied, and $^{99}\text{Tc}^{\text{m}}$ -glucoheptonate was used as the infarct tracer, the ratio between tissue samples and normal myocardial increased with the duration of the ischemic period, which was followed by reflow. This indicates that maybe several different types of necroses may be present in one and the same patient, and definitely in a large population. Such basic difference may account for different pathological uptakes of PYP, and may in fact correlate well with different ECG pictures of ischemia. Other evidence supporting such an argument has been provided by Wackers et al. who found, as did this study, that some patients with UAP on ^{201}Tl -scintigraphy exhibited hyper-

fused areas and even perfusion defects in resting scan. This would suggest that the necrotic events in these patients are not excluded, but that regular parameters are not sensitive enough to detect these necrotic changes. It may therefore not be correct to label the uptake in patients with UAP, as false positive, but rather as positive and then discuss the remaining negatives as false negatives.

The specificity and predictive value of the test increased for all observers if UAP was included as an entity in ischemic heart disease, thus reflecting the possibility of such an event occurring. It may however not be of any great importance whether the patients suffer from UAP or AMI initially, as both these groups of patients seem to require very similar clinical treatment.

Probably false positive PYP scintigrams occurred in valvular disorders and other diseases, which has been described by Karunaratne et al in 1976, Poliner et al in 1976 and Dicola et al in 1976. Karunaratne's group found false positive PYP scintigrams in patients with a mitral valve prolaps syndrome, and here the mechanism behind the uptake is not at all understood. We have previously reported—Lessem and Persson in 1978—that a patient with Chagas disease showed a very strong intense and highly localized uptake—Fig 28—. This patient was later shown to have a totally fibrotic and calcified heart, which was thought to account for the uptake. That patients with Chagas disease can show PYP uptake in the myocardium have later been confirmed by groups from Venezuela, Brazil and Argentina. Muz and his group in 1980 reported myocardial uptake of $^{99}\text{Tc}^{\text{m}}$ -PYP in patients with amyloidosis and related the uptake to the composition of protein in the amyloid deposits in the myocardium.

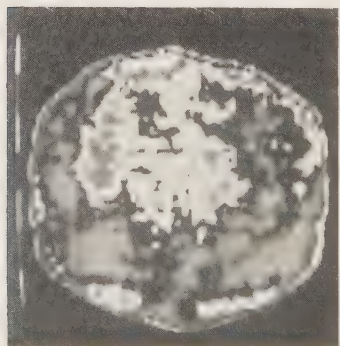


Fig. 28
A false positive PYP scinti-
gram in a patient with Chagas
disease.

Koda et al. in 1976 and Pugh et al in 1976, Stern et al. in 1977 all pointed out that the direct counter current shock in patients with arrhythmias may yield positive PYP uptakes which were of considerable size. A false diagnosis of acute transmural myocardial infarction may be made. Patients exists who are defibrillated due to atrial fibrillation, supraventricular tachycardia, ventricular tachycardia or fibrillation, but who do not have an AMI, and these should therefore be extra carefully examined if PYP is used. In this material the only reason for electrical conversion was ventricular fibrillation in combination with an infarct, except for one patient who had a grade three intense PYP uptake and must be considered a false positive.

Nothing has been found to support the theory that high S-Ca values affect the uptake. However Carr et al. in 1981 have shown that pharmacological and metabolic manipulations of the S-Ca may change the infarct to bone ratio of PYP, in both directions depending on the change in S-Ca.

That a calcified heart however may take up PYP has been seen in one patient, not included in this series, who was referred for a skeletal scintigram due to a methastasing bronchial carcinoma, which was also described by Harford et al. in 1977, and where myocardial uptake was found. When the patient died his heart was found to be full of calcified methastases in the myocardium, supporting findings by Soin et al. in 1975.

Of great importance is that no patients with pericarditis showed any myocardial uptake.

Other false positives in this series were found in patients with no cardiac disorders, but with pulmonary emboli, cerebral hemorrhages, bronchopneumonias to mention a few of the more important. This is in discrepancy with all other published reports on myocardial scintigraphy. These false positives were all found in the low intensity group (1/3), and as such may indicate that these faint scintigrams actually are another expression of the higher reference value and should be considered normal. In one of these a real false positive existed, as has been described previously.

UPTAKE MECHANISMS

The mechanism behind the PYP uptake in necrotic or ischemic cells is not fully understood. At least two complete different mechanisms may be responsible for the PYP uptake. These are the remaining myocardial perfusion and the Ca influx into infarcted areas. Experimental studies by Brunner et al. in 1974 and Buja et al. in 1976, have shown that necroses with contraction band formation, and therefore calcium-deposititis, seem to be a result of the short period of decreased perfusion during the period of injury. At the same time several groups, Shen and Jennings in 1972, D'Agostino and Chiga in 1970 and Jennings and Ganote in 1976 in their studies showed that Ca deposits in irreversibly damaged myocardial cells are to be found in mitochondria and occur in the form of granules which have been shown to consist of hydroxy apatite crystals —Buja et al. in 1976, Jennings and Ganote in 1976—. It has been suggested that the attachment of PYP to necrotic myocardial cells is due to its affinity to these hydroxy apatite crystals. Russel in 1976 speculated on this mechanism and proved that PYP really has the described affinity to hydroxy apatite crystals. Jung and coworkers in 1973 could show similar results, and also that when attached to the crystals, PYP inhibited the aggregation of Ca and phosphates. Several reports by Shelbert et al. in 1975, 1976, have demonstrated that PYP is mainly taken up by irreversibly damaged cells. However they could not in their fetal mouse-heart model show any correlation between the uptake of the infarct imaging agent and the degree of cell injury. The results by Lessem et al. in 1980 in rats with occluded coronary artery, also indicated that the infarcted area accumulated most PYP. Interestingly enough and in contrast with Buja et al. and Martonffy et al. in 1976, Schelbert's group showed that the uptake of $^{99}\text{Tc}^{\text{m}}$ -PYP by irreversibly damaged cells did not at all appear to be primarily related to intramitochondrial Ca phosphate deposits. This supports other observations by Dewanjee et al. in 1976 and by Klein et al. in 1975 both reaching the conclusion that even if ^{45}Ca could be demonstrated in the same areas as $^{99}\text{Tc}^{\text{m}}$ -PYP the Tc chelates most probably were bound to denaturated macromolecules. Coleman et al. in 1977 suggested that $^{99}\text{Tc}^{\text{m}}$ may play a role in the uptake mechanism although he did not further elaborate on this thought. When $^{99}\text{Tc}^{\text{m}}$ -PYP is intravenously injected, an oxidation process occurs. The complex will be oxidized to Tc (IV) state by oxygen. When ischemia is present this

oxidation process may be slowed down, or in necroses almost abolished due to lack of oxygen in the tissue. Such a mechanism would explain the difference in uptake pattern between ischemia and infarction. A recent report that $^{99}\text{Tc}^{\text{m}}$ -sulphur colloids or ^{99}Tc -antimony colloids accumulated in infarcted myocardium in dogs, seem to indicate that Tc may play an important role, and that colloid particles may pass through the damaged endothelial cells of the walls of the blood vessels, and be retained by intact basement membrane (Swanson et al. 1980).

No studies have been performed trying to block the uptake of $^{99}\text{Tc}^{\text{m}}$ -PYP with a Ca antagonist. Several slow channel blocking agents have been investigated in the treatment of coronary artery disease, but no experiments combined with myocardial scintigraphy has been performed. It is theoretically possible that PYP uptake may decrease in size and intensity in patients pretreated with a Ca blocking agent early in the course of an AMI.

Not even the experiments by Hansson et al. in 1980 in labelling cardio-selective beta blockers yielded any information as to why PYP was accumulated in damaged myocardial cells. There seems to be no connection with catecholamines and a PYP accumulation.

Due to the findings that both UAP and AMI do take up the tracer, discussions are to be found in the literature whether the remaining perfusion or not plays an important role in the uptake. Walsh et al. in 1975 conclusively demonstrated that the uptake of phosphate labelled with $^{99}\text{Tc}^{\text{m}}$ was flow dependant.

Buja et al. in 1975 could also show that the uptake was located in the peripheral zone rather than in the central necrotic areas, and thus perfusion seemed to be of importance. A key determinant of PYP accumulation seems to be the status of the remaining regional myocardial perfusion after occlusion, and a flow reduced to 95% of the normal is not accompanied by any uptake. Marttonfy et al. in 1976, proved that transient occlusion yielded stronger positive uptake than did permanent occlusion, something Zaret et al. in 1976 disputed when

they found strong uptakes in the necrotic areas using both ^{43}K and $^{99\text{Tc}}\text{m-PYP}$ in the same patients. Cells exhibiting neutral lipid accumulation, are often found in the peripheral infarct zone, and the uptake of PYP is highest in this zone. Long and coworkers' studies from 1980, however seem to support that reperfusion necrosis do accumulate more PYP than does permanent occluded necrosis. Parkey et al. in 1977 summarized these two views and they came to the conclusion that residual of collateral blood flow is a vital determinant of the uptake. Coleman et al. in 1977 and Marcus et al. in 1976 reached the same conclusions when they found that segmental myocardial perfusion was an important determinant of $^{99\text{Tc}}\text{m-PYP}$ accumulation by myocardial areas containing necrotic cells.

PROJECTIONS AND TIME INTERVALS

The number of positive uptakes varied much in the different pictures. The picture with most information was the AP 60, followed by the AP 90, while less information was collected from the two LAO pictures. The scintigrams were recorded by three people, one physician and two technicians, all using the same technique. They were thereafter put in order from 1 to 635 by an unbiased technician, not consecutively, and this procedure does not explain why the LAO pictures gave less information. The reason for the two anterior posterior pictures giving most information, may instead be that it was easier for the observers to register changes in a frontal picture. It is also possible that these two pictures were registered at the beginning and ending of the examination, and were most apart in time. Collecting 400,000 counts took several minutes, and the changing of collimator position required another few minutes. Very conflicting reports however, have been published as to when the optimal scanning time is, and everything from 60 minutes to 180 minutes has been suggested. The general rule has been to avoid a later scanning time after injection when the bone uptake is becoming more prominent.

Although the majority of patients were examined within 48 hours after onset of symptoms, myocardial scintigraphy with ^{99}Tcm -PYP did not give a clinical diagnosis of AMI earlier than other used laboratory methods. Diffuse positive and negative scans occurred earlier during the time course than other laboratory methods yielded a diagnosis. This is in accordance with other published reports (Willerson et al. 1977, Tetelman et al. 1977). The predictive value of a negative test was high and very reliable. Such a finding is of importance because it may allow the physician to initiate alternative treatment for the proper disease at an earlier stage, or maybe even dismiss a patient earlier from the ward or the emergency room when other initial tests also are negative (Lessem 1978) and save substantial amounts of costs.

LOCALIZATION

The ECG localization was as stated previously generally found to be smaller than the scintigraphically determined. This has also been shown previously (Parkey et al. 1976, Botvinick et al. 1977). It seems possible to assume that some of these scintigraphic uptakes may be accounted for by the oedema around the infarcted area. Support for this can be found in the fact that perfusion defects on TI scintigram tend to decrease over the time of the acute course of the infarct without any pharmacological or surgical interventions. Another reason maybe that the gamma camera is overlooking a larger area than the single ECG electrode, and comparison should then preferably be made with extensive ECG maps. Autopsy data (Buja et al. 1976) showed similarity in the size of the PYP uptake area and the necrotic areas in dogs with induced infarcts.

Some localizations ought to be easier to detect with a gamma camera than with a regular ECG. One such area where this seems to be true is the right ventricle. In this study no right ventricular ECG leads were used, and Erhardt in 1976 suggested that this was necessary if the majority of the right ventricular infarcts are to be diagnosed. This seems to be an area where scintigraphy with ^{99}Tcm -PYP is superior, as has been shown by Busseman-Sokole et al. in 1977, and Legrand et al. in 1981. Another such area is the septum where one can assume that this often is a part of a large infarct. The assumption seems to be

verified by the fact that scintigraphy often localized an uptake as partly septal, even if it extended itself to either the apex or was more anterior in its localization.

PROGNOSTIC VALUE

In our study we could not demonstrate any prognostic value of myocardial scintigraphy with $^{99}\text{Tc}^{\text{m}}$ stannous PYP. This contrasts this report to reports by Olsen et al. in 1978 and Parkey et al. in 1977. The only pattern that had any prognostic indication was the dough-nut pattern which indicated serious complications and fatality but not the type of the complication. These data are in concordance with the report by Olsen et al. in 1978 and recently by Ahmad and coworkers in 1981. Why the PYP accumulation takes the form of a dough-nut is unknown, but Buja et al. in 1976, 1977, 1978 have shown on several occasions, especially in dog experiments, that this probalby is due to large infarcts, where no reserve blood flow to the centre of the infarcts exists at all. No deposition of hydroxy apatite crystals occur, and secondary to this there is no possibility for a PYP accumulation. Similar results were found by Horner et al. in 1980 from their experiments with isolated heart preparation. Due to the size of these infarcts, usually damaging the better part of the myocardium, the general prognosis is over all poor. The PYP scintigraphy indicates this, but there is an aparent selection of which patients that are going to show this pattern, and the mechanism behind this selection is unknown. One may speculate that the cause of death may play a major role, or that the infarcted area's metabolism is influencing the pattern of the uptake. No studies to this effect has so far been carried out. The only indications come from different positron and metabolic studies by Stöckler et al. in 1980 and Evangelou et al. in 1977, Weiss et al. in 1975, 1977, all suggesting a fast turn over in the ischemic metabolism.

The uptake in patients with UAP had no prognostic value as to who are going to develop an infarct or not, and this is a finding which is somewhat disappointing. It was hypothesized that one would be able to use different uptake patterns in UAP and acute infarction to determine what would happen in patients with UAP. So far no studies have shown that if the uptake remains for a long period of time this would have any importance. No indications as to the clinical outcome of these

patients were found in this material. In the patients dying after the hospitalization period no definite uptake pattern was seen, but some of them had PYP accumulation of intensity two. It may be worthwhile observing such patients closer with a more frequent clinical follow-up to prevent a fatal episode.

It can be said that PYP in general is a poor prognostic indicator but that some prognostic information may be obtained.

TI STUDY

The results presented in this study showed a higher diagnostic accuracy for the ^{201}Tl myocardial scintigraphy in patients with AMI when read by observer B. This is in accordance with a great many other studies by Wackers et al in 1975, 1976, 1978, Strauss et al. in 1976. Wackers has shown that the TI-scintigrams yielded 100% diagnostic accuracy if the patients were examined within 6 hours after onset. In contrast to other studies we found negatives in patients with acute transmural myocardial infarction. This could be due to that they were smaller than the enzyme values gave reason to believe. Another plausible explanation may be found in the spasm theory that Maseri et al. in 1977 have put forward. It might be that an infarction based on a temporary coronary spasm which suddenly was relieved, would not lead to a detectable perfusion defect at all times. In order to investigate this it would have been very valuable if these patients would have been reinvestigated or provoked with ergonovine. A further reason may be that the oedema around the infarcted area may have gone in regress, and that then no definite detectable perfusion defect remained. Heinz et al. in 1980 also showed that if ^{201}Tl was contaminated with a lot of ^{202}Tl or ^{200}Tl the quality of scintigrams decreased substantially, and such a contamination may account for some false negatives.

As well as others-Wackers et al. in 1978—, we found that patients with UAP could show perfusion defects or hypoperfused areas. This is not surprising in the light of the positive PYP uptakes, which again leads one to speculate if not some of these uptakes and defects actually ought to be explained by myocardial necrosis of one type or another as Baroldi in 1976 has suggested.

The possibility of detecting coronary insufficiency by an exercise TI study has been widely used, and proven to contribute knowledge besides the stress ECG of diagnostic value (Zaret et al. in 1977, Ritchie et al. in 1979, Berman et al. in 1978, 1979, McLaughlin et al. in 1977, Hamilton et al. in 1978, McKillop et al. in 1980). The mechanism of decreased oxygen supply in a situation of increased demand, may of course be explanatory for the mechanism behind the perfusion defects in patients with UAP. Two from each other distinct pathophysiological mechanisms may then account for the presence of perfusion defects or hypoperfused areas in patients with UAP. If these two mechanisms also can account for the PYP uptake has been discussed elsewhere.

Old infarcts have been known to yield perfusion defects (Wackers et al. in 1977, S-E Svensson et al. in 1978). This in turn indicates that ^{201}Tl myocardial scintigraphy can not be used successfully and safely in patients with chest pain of unknown origin. It is even questionable if ^{201}Tl scintigraphy can be utilized on patients believed to have their first infarct, unless tomography can be obtained, as the patient may have had a silent infarct previously, not resulting in any ECG changes.

If combining PYP examinations with TI-examinations it seems to be clear that the diagnostic yield is higher, which is similar to reports by Berger et al. in 1977, Dressler et al. in 1978, Henning et al. in 1976, Parkey et al. in 1975, 1976. Berger's group found discordant size estimation of an infarcted area with $^{99}\text{Tc}^{\text{m}}$ -PYP and ^{201}Tl . A combined scintigraphy examination will probably only be needed in very rare cases when no diagnosis can be obtained by other means, but there it is essential for the treatment of the patient that a correct diagnosis is arrived at.

GENERAL SUMMARY

This investigation has shown that in animals with induced myocardial infarction, PYP is taken up mainly by necrotic cells and less by other surrounding myocardial ratios. The infarct to border zone ratio was high in both in vivo and in vitro examined hearts. The maximum uptake of PYP in rats with ligated LAO occurred 24 hours after ligation. It was also shown at least in rats, that PYP is eliminated with the same rate as polymorpho nuclear cells are. It must be remembered that the rat is different from other species, where the PYP-uptake has been known to be present for a longer period of time after the acute event. For human beings this phenomenon has also been shown by this investigation.

In patients with AMI, PYP was shown to accumulate in this study, but to a lesser degree then in previous reports. Several other diseases also showed myocardial accumulation of PYP. The amount of false positive and false negative studies were larger in the present investigation then in other reports. A wide range of interobserver variability was found, when judging intensity of each picture. This variability was smoothened out when ROC curves were plotted.

In this study the intensity grade 2 would be the closest to equalling an AMI, but the same was found for other diseases. At this intensity the sensitivity would be vary between 20–1%, while the specificity would vary between 95–99%. Our study showed that intensity grade 2 was to widely defined, and when an imaginary intensity grade 1,4–1,6 was chosen the sensitivity ranged between 65–70% and the specificity between 60–65%. At that intensity level the efficiency was 66%, and the predictive value of a positive test 72%. This is too low to defend myocardial scintigraphy as a replacement over ECG and enzymes, but high enough to render it a place as an additional tool.

It was found that UAP does take up PYP. The intensity of this uptake overlapped with the intensity of the PYP uptake in patients with AMI. No patients with UAP had the highest intensity grade (3/3) uptake in a serie of four scintigrams. The weighted score and IAS never reached the maximum levels in patients with UAP. The overlap in the low intense group makes it difficult to separate the two groups by intensity grading only. A tendency to lower intensity did exist. For the same imaginary

intensity grade 1,4–1,6, the sensitivity for UAP would vary between 58–48% and the specificity between 40–52%. The PYP uptake in patients with UAP was diffusely localized over the whole myocardial area, whereas it in patients with AMI was more well defined in its localization. This was a finding all observers agreed on. The ROC curves were also separated for all observers in these two diseases. It may therefore be possible to obtain information about the natural history of UAP with this method. Myocardial scintigraphy with $^{99}\text{Tc}^{\text{m}}$ -PYP may, although with difficulties, be used in differentiating UAP and transmural AMI. The possibility of an diffuse low intense PYP myocardial uptake representing a patient with UAP is greater then that it represents a patient with transmural AMI.

We have no indications in this material that the uptake pattern was significantly attached to any patient prognoses. PYP scintigraphy as a static examination can not be used for prognostic purposes. No pattern was indicative of the progress of the disease, nor of the forthcoming death or later reinfarction.

No definite data has been presented to show that the PYP uptake in AMI is an earlier infarct responder then other available routine methods.

The previous experience in reading myocardial scintigrams, played a minor role in the interobservervariability. The observers with the most experience read more scintigrams as having the highest intensity, but that differance was rather small. Using a majority descision a great interobserver variability as to what was determined as an infarct by positive scintigrams, was found, ranging from 74% to 33% for true positives and from 25% to 3% for false positives.

CLINICAL IMPLICATIONS

In general myocardial scintigraphy with $^{99}\text{Tc}^{\text{m}}$ stannous PYP in a population of patients with suspected acute infarctions added a new dimension to the diagnosis of AMI, but is of less value and shows less specificity and sensitivity than initially claimed. The reasons for this have been thoroughly discussed. Therefore it seems advisable to conclude this monograph by recommending that the use of myocardial scintigraphy should be in situations where no other instrument give the proper diagnosis or when the suspicion is clinically strong but can not be otherwise verified.

We have shown in a large patient population that myocardial scintigraphy with $^{99}\text{Tc}^{\text{m}}$ -PYP is an additional tool in diagnosing AMI.

We believe that myocardial scintigraphy with $^{99}\text{Tc}^{\text{m}}$ -PYP can be used in cases where other parameters seem to fail, e. g. in patients with left bundle branch block, other ECG abnormalities, pacemaker ECG:s, liver diseases, muscular diseases and although it has not been within the aim of the present study to examine patients post cardiac surgery, it has been reported that PYP scintigraphy is of great value in that patients population, Howe et al. in 1976, Klausner et al. in 1977.

The referred literature also recommend that the examinations are combined with hemodynamic measurements that can be made on the same injection so that more valuable information can be obtained. It seems well advised to recommend each institution embarking on $^{99}\text{Tc}^{\text{m}}$ -PYP myocardial scintigraphy, to define its own intensity grade and its sensitivity and specificity. From this investigation it is apparent that the levels for the intensity grade made vary with observers, and it is therefore advisable that the observers interpreting the scans should be the same in an institution.

Myocardial scintigraphy with $^{99}\text{Tc}^{\text{m}}$ -PYP should be performed within 48 hours post onset, and a mobile gamma camera should be used, also recommended by Rose in 1976, Dymond et al. in 1977. Computer manipulations of the analogue pictures may be of value and it is then possible that all positive scintigrams may belong to patients with ischemic heart diseases. From this and other studies referred to, it can

be concluded that AMI exhibits an uptake of PYP, which can be distinguished from the one present in UAP. We feel that this finding is of great clinical importance and can be used for selecting proper treatment.

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